PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GARY L. CLAYMAN

Serial No.: 08/758,033

Filed: November 27, 1996

For: METHOD AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Group Art Unit: 1632

Examiner: K. Hauda

Atty. Dkt. No.: INRP:041/HYL

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REPLY BRIEF

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[1] Exhibits A-P were submitted with the Brief on Appeal

U	-	Planchon *et al.* 1992
V	-	Welters *et al.* 1999
W	-	Vingerhoeds *et al.* 1996
X	-	Mourad *et al.* 1996
Y	-	Liu *et al.* 2000
Z	-	Johansson *et al.* 1991
AA	-	First Zumstein Declaration
BB	-	Ueyama, 1987

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BOX AF
Hon. Asst. Commissioner of Patents
Washington DC 20231

Dear Sir:

This Reply Brief is filed in response the Examiner's Answer, mailed on February 17, 2000, regarding the above-captioned application. No fees are believed due in connection with this filing. Also accompanying this brief is appellant's Request for Oral Hearing, along with the required fee. Should appellants' fee be missing, or should any other fees be due, the Commissioner is authorized to debit Fulbright & Jaworski Deposit Account No. 50-1212/10012436/01973. The Board is respectfully requested to consider the following additional remarks prior to oral hearing.

I. Status of the Claims

Claims 1-25 were filed with the original application. Claims 26-145 have been added. Claims 15, 21-25, 78 and 79 were canceled in the first response, and claims 33-35, 69-72, 104-107, 133-136, 140, 141, 144 and 145 were canceled in an amendment filed concurrent with the Brief on Appeal. Thus, claims 1-14, 16-20, 26-32, 36-68, 73-77, 80-103, 108-132, 137-139, 142 and 143 are pending and appealed.

II. Status of the Amendments

Appellant filed an amendment with the Appeal Brief requesting cancellation of claims 33-35, 69-72, 104-107, 133-136, 140, 141, 144 and 145, and the introduction of minor amendments to various of the claims. This amendment was entered.

Appellant is filing another amendment, concurrent with this Reply Brief, requesting cancellation of claims 38-68, 73-77, 80-103, 108-132, 137-139, 142 and 143. This amendment is provided merely to simplify the issues on appeal and in no way represents and acquiescence to the rejections of record.

III. Issue Remaining on Appeal

Are claims 1-14, 16-20, 26-32, 36 and 37 obvious over Cajot *et al.* (Exhibit E) taken with Wills *et al.* (Exhibit B) or Liu *et al.* (Exhibit A), in view of Zhang *et al.* (Exhibit C) or Bramwell (Exhibit D)?

IV. Reply

The examiner has maintained the rejection of claims 1-14, 16-20, 26-32, 36 and 37 obvious over Cajot taken with Wills or Liu, in view of Zhang or Bramwell. Given appellant's cancellation of claims 38-68, 73-77, 80-103, 108-132, 137-139, 142 and 143, this now is the only issue remaining in this appeal.

A. Summary of the Brief and the Examiner's Answer

The appeal brief addressed a number of reasons by the rejection of claims 1-14, 16-20, 26-32, 36 and 37 could not be found "obvious" over the cited references. First, appellants argued that extrapolation from *in vitro* to *in vivo* studies is problematic at best. Second, with respect to Cajot *et al.*, it was argued that these studies are unrelated to the actual therapy of human tumors, and are technically flawed. Third, the Baker *et al.* (Exhibit J), Casey *et al.* (Exhibit K), Katayose *et al.* (Exhibit F) and Srivastava *et al.* (Exhibit G) references were said to evidence confusion in the art. And fourth, appellants provided experimental data showing surprising and unexpected results flowing from practice of the present invention.

In turn, the examiner has argued that "there has been no rejection on the record that extrapolating from *in vitro* to *in vivo* inhibition of tumor cells expressing p53 is problematic or unpredictable." Issues of extrapolation are said to be fact specific, and in the instant case, Wills and Liu establish that extrapolating from *in vitro* to *in vivo* is appropriate.

The examiner also argues that Baker *et al.* (Exhibit) and Casey *et al.* (Exhibit) do not, as alleged by appellants, evidence confusion in the field. To the contrary, the examiner argues that

these references support the notion that overexpression of p53 can inhibit growth of a wide variety of cells, including p53-positive tumor cells.

Next, the examiner disputed that Cajot is flawed, arguing that because SV40 "seems to be the least affected" by p53, the skilled artisan would be motivated to use this promoter in studies and, further, that the limitations on Cajot's data are both "misstated and misconstrued giving an interpretation that the art does not teach."

Finally, the examiner rebuffs appellant's clinical data on the ground that the statistical comparison between p53-positive and p53-negative cells is invalid in that the groups have different sample sizes. Moreover, even accepting the statistical significance at face value, the examiner argues that the extent of the "surprising result," being limited to that tumor type tested (head & neck), is narrower than the present claims.

In the following pages, appellants will provide a rebuttal to each of these specific points, thereby again refuting every basis for the rejection.

B. It is Necessary to Focus on the Claimed Invention in Assessing Obviousness

At the outset, appellants would like to draw the Board's attention to the specific language of the only remaining independent claim, claim 1. It is important, is exploring the remaining rejection, to focus on the recited claim elements. If properly interpreted, appellant submits that the present claims clearly are not suggested by the cited reference. Claim 1 reads as follows:

> A method of inhibiting growth of a p53-positive tumor cell in a mammalian subject with **a solid tumor** comprising the steps of:

(a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and

(b) directly **administering said viral expression construct to said tumor *in vivo***, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth,

wherein said tumor comprises cells that express a functional p53 polypeptide.

(Emphasis added). Thus, the following elements of the claim must be suggested by the prior art: first and foremost, one must show treatment of a solid tumor in a mammalian subject; second, the treatment must involve the use of a p53-encoding polynucleotide; third, tumor cells within the tumor must be p53 positive, *i.e.*, express a functional p53 polypeptide; and fourth, the treatment must be demonstrated as inhibiting tumor cell growth.

Turning to the art, the examiner has cited the following. First is Cajot, which even read as broadly as the examiner argues, shows only *in vitro* studies with p53-positive tumor cells. Next is Wills and Liu, both of which are cited for *in vivo* animal studies, but only show studies with p53-deficient cells. Finally, Zhang and Bramwell address combination therapies. The propriety of this combination, including assumptions that must be made in order to permit it, are central issues of this appeal. As will be explained, the combination is improper, as is the remaining rejection.

C. There Is No Reasonable Basis for Extrapolating from In Vitro to In Vivo

As just stated, one key issue here is whether the examiner's combination of Cajot with Wills and Liu, as framed above, is proper. If not, the rejection must fall. It is appellant's

position that this combination is ***not*** permissible given the legal requirements for any obviousness rejection, which include (i) detailed enabling methodology for practicing the claimed invention, (ii) a suggestion for modifying the claimed invention, and (iii) evidence suggesting that the invention would be successful if made. *In re O'Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988). More specifically, appellant submits that the differences between *in vitro* and *in vivo* studies, especially when simultaneously making the leap from treating p53-negative to p53-positive cells, simply is not warranted.

It is appellant's position that the cited art that relates to p53-positive cells, which shows only *in vitro* data, cannot be relied upon to predict what would happen in an *in vivo* context. The limitations of *in vitro* are manifest, and so well supported in the art that it is unnecessary to recount them here. In fact, the first Office Action addressed limitations on gene therapy, and specifically discussed the shortcomings of delivery and expression of transgenes *in vivo*:

> ... The unpredictability of gene therapy and vector targeting is supported by the teachings of Culver *et al.*, Hodgson *et al.* and Miller *et al.* Culver *et al.*, reviewing gene therapy for cancer, conclude that the "primary factor hampering the widespread application of gene therapy to human disease is the lack of an efficient method for delivering genes *in situ*, and developing strategies to deliver genes to a sufficient number of tumor cells to induce complete tumor regression or restore genetic health remains a challenge" (page 178). Hodgson discusses the drawbacks of viral transduction and chemical transfection methods, and states that "[d]eveloping the techniques used in animal models, for therapeutic use in somatic cells, has not been straightforward" (pages 459-460). Miller *et al.* also review the types of vectors available for *in vivo* gene therapy, and conclude that "for all the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances ... targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1).

First Office Action, pages 5-6.

The art is replete with examples of cancer treatments that showed promise *in vitro* only to fail *in vivo*. For example, Planchon *et al.* (1992) showed that butyrate derivatives inhibited growth of breast cancer cell monolayers *in vitro*, but failed to affect the rate of tumor growth *in vivo*. Exhibit U. Welters *et al.* (1999), in examining the effects on cisplatin in head & neck cancers, found a lack of correlation between studies on *in vitro* tumor cell lines and *in vivo* tumors. Exhibit V. Vingerhoeds *et al.* (1996) similarly compared the effects of doxorubicin on ovarian carcinoma cells and found that *in vitro* inhibition was not observed *in vivo*. Exhibit W. Mourad *et al.* (1996) showed that high doses of vitamin A inhibited head & neck and lung cancers *in vitro*, but showed no similar effects *in vivo*. Exhibit X. Liu *et al.* (2000) disclosed that, *in vivo*, secretion of TGF-β correlated with resistance to tumor therapy, while no correlation was observed *in vitro*. Exhibit Y. Finally, Johansson *et al.* (1991) demonstrated that a murine monoclonal antibody inhibited cancer cells *in vitro*, but that *in vivo* inhibition was limited to two days after inoculation into animals, hardly a clinically relevant situation. Exhibit Z.

A more extreme example was provided in appellant's brief, where it was noted that Srivastava[2] disclosed "[a] recent study [which] described an intriguing result in which an adenovirus-p53 expression vector did not inhibit the *in vitro* growth of a metastatic variant of LNCaP cells; however, the growth of these cells was inhibited *in vivo*.[31]" This is additional evidence that there is ***considerable*** unpredictability as to whether *in vitro* results will be the same as those in an *in vivo* environment.

[2] Appellants note that the examiner has withdrawn a rejection based on this reference in light of a previously submitted declaration under 37 C.F.R. §1.131.

However, the examiner attempts to circumvent this argument on the basis that the p53 field is different, namely, in that there was a demonstrated correlation between *in vitro* studies and *in vivo* animal models at the time the present application was filed. However, any such correlation related to p53-***deficient*** cells. Thus, it would be necessary, in relying on this showing, to have some basis for equating the treatment of p53-positive and p53-negative cells. It is clear that the conventional thinking, as of appellant's priority, was that p53 ***replacement*** was capable of restoring growth regulation to cells lacking that function. It was far less clear that p53 ***supplementation*** would have any real benefit.

As argued previously, there was considerable confusion in the field regarding gene therapy p53-positive tumors. For example Katayose[3] stated that "tumor cells that were null for p53 prior to infection ... and tumor cells that expressed mutant endogenous p53 protein ... were more sensitive to AdWTp53 cytotoxicity than cells that contained the wild-type p53 ..," and that "these studies indicated that an adenovirus vector expressing wild-type p53 is markedly cytotoxic ***to tumor cells that have null or mutant p53 expression*** ..." (emphasis added). In addition, the last line of the abstract summarizes the authors conclusions: "These data suggest that endogenous p53 status is a determinant of AdWTp53-mediated cell killing of human tumors." The clear inference is that only p53 null or p53 mutant tumor cells are killed by AdWTp53, not tumor cells that are WTp53.

Katayose indeed actually teaches away from treating p53-positve tumor cells with p53 expression vector. In the Discussion on page 896, first column, second paragraph, it is stated

[3] See footnote 2.

that "There are several possible mechanisms by which high expression of wild-type p53 results in apoptosis in tumor cells devoid of p53 or expressing mutant p53, but not in tumor or normal cells expressing wild-type p53". Thus, Katayose is itself stating quite clearly that expression of wild-type p53 would not be expected to effect apoptosis in a tumor which expresses wild-type p53.

The following additional comments also illuminate what the skilled artisan would take away from Katayose:

> "As shown in Fig. 3, *A* and *B*, infection of H-358 and MDA-MB-231 [p53 null and mutant, respectively] cells with AdWTp53 completely inhibited cell growth .... In contrast, MCF-7 cells [p53 positive] continued to proliferate although at a slower rate than control cells ...."

Page 892, right hand column.

> "It appears that cells that express wild-type p53 were 5-250 times more resistant to the AdWTp53-mediated inhibitory effect on cell growth when compared with cells expressing no *p53* or mutant *p53*."

Page 893, right hand column.

> "These results indicate that tumor cells null for *p53* or expressing an endogenous mutant *p53* undergo apoptosis following exposure to AdWTp53, whereas tumor cells or normal cells expressing wild-type p53 are resistant to apoptosis."

Page 895, right hand column.

> "... [O]verexpression of wild-type p53 induced programmed cells death (apoptosis) of tumor cells devoid of wild-type p53 or expressing endogenous mutant *p53*, but not in tumor or normal cells expressing wild-type p53."

Page 896, left hand column.

These passages clearly indicate that the Katayose reference cannot be read as providing sufficient motivation for treating p53-positive cells. To the contrary, the reference suggests

the opposite, that p53-positive cells are far less susceptible to such treatments. Unfortunately, the examiner failed to provide any response to these arguments.

Srivastava also provides an insufficient basis for suggesting that one should ***clinically*** treat p53-positive cells with a p53 expression construct. In fact, they state that "in agreement with the previous observations,[18] we also did not detect a growth inhibitory effect of AdWTp53 on breast cancer cells, MCF7 containing endogenous wt p53 (data not shown)." No clearer statement to counter the examiner's position could be imagined.

The examiner's response to this argument glosses over the MCF7 issue and turns instead to DU145- and PC3-derived cells as showing predictability for *in vivo* applications. This misses the point entirely. MCF7 has wild-type p53; the other cells lines do not. The reference, therefore, suggests to the skilled artisan that p53 gene therapy of p53-positive cells will ***not*** be successful. The Answer fails entirely to address this issue.

Turning to Baker and Casey, the examiner has provided nothing to rebut appellant's arguments regarding the latter reference. As for Baker, the examiner argues, based on the following equivocal passage, that it actually supports the rejection:

> The transfection and expression results of Table 1 and Fig. 2A suggest that cells at the premalignant stages of tumor progression (VACO 235) may be less sensitive to the inhibitory effects of wild-type p53 than malignant cells (SW480, SW837 and RKO). This hypothesis is consistent with previous results that suggest the wild-type p53 is less inhibitory to the growth of normal rat embryo fibroblasts than to their oncogene-transfected derivatives (8). This sensitivity may only be relative: expression of the wild-type gene at high concentrations might be inhibit the growth of any cell type, including non-neoplastic cells, by overwhelming normal regulatory processes such as phosphorylation.

The examiner's position is fanciful. Somehow, the examiner reads this passage as suggesting gene therapy of p53-positive cells, despite the facts that: (a) the passage notes that premalignant (wtp53) cells are ***not*** as sensitive to p53 transgenes; and (b) normal fibroblast cells (wtp53) are unaffected by p53 trangenes. Worse yet, the last sentence is highlighted by the examiner, but says nothing about a clinical application. Non-specific inhibition of ***any*** cell type, which is all that is proposed in this emphasized statement, would be absolutely ***useless*** since ***all*** cells would be killed, not just tumor cells. By analogy, hydrochloric acid would be a useful chemotherapeutic.

Thus, it is submitted that there is no basis for extrapolating from the *in vitro* studies of Cajot to the *in vivo* studies of Wills and Liu. This is based not only on the well established limitations of *in vitro* systems, but on the clear confusion in the field, as evidenced by Baker, Srivastava, Katayose and Casey.

D. The Cajot Reference is Flawed

Another problem with the rejection is its absolute reliance on Cajot. As has been argued extensively in prior submissions, the Cajot reference cannot be taken on its face due to serious technical flaws in the experimental design. In particular, appellant has provided evidence that the SV40 promoter used in the Cajot studies is down-regulated by p53, thereby skewing the results in such a way that the skilled artisan would not rely on the data report therein.

In the Answer, the examiner replied by stating that Subler *et al.* (Exhibit L), which was cited as support for the down-regulation of the SV40 promoter, disclosed that "[t]he SV40 early

promoter seems to be the least affected under our assay conditions." While acknowledging that Subler taught inhibition of a variety of cellular and viral promoters, it is argued that those of skill in the art "would be motivated to use the SV40 early promoter to get the highest level of expression possible." Nonetheless, whatever motivation existed, the data flowing from these experiments was flawed and, hence, so would the conclusions drawn therefrom.

Another serious technical flaw exists within Cajot. As discussed in the declaration of Dr. Lou Zumstein, the nude mouse studies described in Cajot. were not true *in vivo* studies. The lung tumor cell lines at issue there were transfected with the p53 vector *ex vivo* (*in vitro*) and only ***then*** injected into the nude mice. Such an assay is not a true *in vivo* assay since one is not establishing tumor *in vivo* first, and then treating the established tumor.

Where one employs an *ex vivo* assay such as was employed by Cajot, there is no test for the effects of the therapy on the tumor *in situ* in the patient's body. Many questions remain unaddressed by such an experiment -- *e.g.*, is the vector capable of penetrating and entering the tumor cells *in situ*; does the therapy have an effect on the tumor mass when the tumor mass in actively growing in an animal (as opposed to mere cells in a test tube); is there sufficient distribution of the vector to cells of the tumor, and sufficient expression within those cells to effect a noticeable growth inhibitory effect; can the material pass through the extracellular matrix that comprises the tumor mass; are there extracellular proteins in the tumor millieu that might block uptake, *etc.*? Studying the effect of a gene such as p53 on cells *in vitro* tells one little about the ability of a gene to work as a tumor suppressor gene in the clinic, and would not be

relevant to the claims at issue in this appeal, which are directed to direct administration to a tumor *in vivo*.

The proper design of a nude mouse tumor assay, where one desires to duplicate an *in vivo* anticancer therapy, is set forth in Ueyama, "Utilization of Nude Mice in Research on Human Cancer," in Animal Models: Assessing the Scope of Their Use in Biomedical Research, 1987 (Exhibit BB). Ueyama extols the importance of using the nude mouse assay in assessing anticancer agents and points out that the assay involves ***first*** establishing the cancer in the nude mouse by xenotransplantation, and ***then*** treating the resultant tumor with the anticancer agent *in situ* (p. 289). As with gene therapy, it would make little sense to treat the cancer cells in a test tube with the anticancer drug and then transplant the cells into the mouse if one desires to test the clinical effect of the drug on the tumor *in situ*.

The one study presented by in Cajot in nude mice (albeit using the flawed *ex vivo* rather than *in vivo* therapy as pointed out above) would not support a conclusion that p53 would be an effective therapy against wild-type p53 tumors. In contrast, the Cajot study would argue ***against*** such a conclusion. In the nude mouse study shown in Cajot's Figure 3, the only transfectant purported to be "wild-type" was the cell line designated "X833.W2." However, it is clear from Figure 1C and from the text that "X833.W2" is not a wild type clone at all -- it expressed a mutant p53: "X833.W2 was shown by Western blot analysis to express what appears to be a mildly truncated form of the p53 protein". Page 6958, col. 2.

Furthermore, there are 17 other allegedly wild-type p53 transfectants reported (see Figure 1A). Inexplicably, no nude mouse growth results are shown with any other wild type p53 transfectant. Yet, the text explicitly discloses that such growth assays ***were*** conducted with at least 5 other wild-type p53 transfectants but that these transfectants were ***not*** growth inhibited -- that these wild type transfectants were ***failures***. See p. 6958, col. 1, last full sentence, and middle of col. 2.

Thus, from the studies reported in Cajot *et al.*, it is evident that out of at least 18 different wild-type p53 clones that were analyzed, only one was reported to exhibit some form of growth suppression -- the other 17 were not reported to show any growth suppression. And this one clone that was allegedly growth suppressed, X833.W2, in fact expressed only a mutant p53. The only reasonable scientific conclusion that one can draw from these studies is that the introduction of the p53 gene into the wild-type p53 lung cancer cell line was ***not*** effective at reducing the growth rate of the lung cancer cells in 17 of the 18 purported "wild-type" transfectants, and the one instance where there did appear to be growth suppression, such suppression could not be attributed to wild-type p53 expression.

E. The Provided Clinical Comparison is Relevant

As submitted in the Brief on Appeal, current clinical data supports a conclusion of surprising and unexpected results in the context of the clinical application of the present invention. The clinical data further provides support for the conclusion that when applied in a clinical context, the invention is applicable in the treatment of wild-type p53 expressing tumors virtually to the same degree as in the context of non-wild-type p53 expressing tumors. This is most surely a surprising and unexpected result.

The examiner argues that, since the p53-positive and p53-negative groups compared in this analysis were not of equal size, there can be no statistical relevance. However, appellant provided these comparisons not for statistical purpose but as a general response rate comparison. Moreover, since both groups were presented at a ratio (responders v. total tested), the sample size for each would not matter. In sum, these data show a general trend for response rates between p53-positive and p53-negative tumor cells to be about the same. This is surprising.

F. Other In Vivo Studies Indicate That the Claims are of Appropriate Scope

The examiner has, in previous actions, focused on the fact that appellant's claims cover "any and all" tumor types, but that only head & neck cancer data are provided. However, other studies evidence the broad applicability of the present invention. For example, one study involving an *in vivo* model of adenoviral p53 therapy of prostate tumors used wild-type p53 prostate cancer cell line LNCap to establish tumors in the prostates of nude mice. Once established, the prostate tumors were treated with intra-prostatic injection of adenoviral p53. Since it is difficult to measure tumor volumes in this model, and since LNCap cells produce PSA, serum PSA levels, an accepted surrogate for prostate tumor volume, were measured. Adenoviral p53 treatment of this p53 wild-type tumor caused significant reduction of PSA levels, evidencing a reduction in the growth of these p53 wild-type tumors. The actual data is shown in Figure 1 attached to the Zumstein Declaration.

In another study, involving an *in vivo* model of adenoviral p53 therapy of lung tumors, the wild-type p53 lung cancer cell line A549 was used to establish subcutaneous tumors in nude mice. Once established, tumors were treated with intra-tumoral injection of adenoviral p53.

Adenoviral p53 injection into this p53 wild-type tumor caused a significant delay in the rate of tumor growth. The data is shown Figure 2 attached to the Zumstein Declaration.

In yet another study, involving an *in vivo* model of adenoviral p53 therapy of cervical tumors, the wild-type p53 cervical cancer cell lines SiHa and MS751 were used to establish subcutaneous tumors in nude mice. Once established, tumors were treated with intra-tumoral injection of adenoviral p53. Adenoviral p53 injection into both of these p53 wild-type tumors caused a large and significant delay in the rate of tumor growth. The data for SiHa is shown in Figures 3A (single Ad-p53 injection 25 d after tumor cell implantation), 3B (3 injections of Ad-p53 at 25 d post implantation) and 3C (6 injections of Ad-p53 post implantation), attached to the Zumstein Declaration.

Each of these studies shows why the present invention is applicable to a wide variety of cell types and, similarly, why the present claims should not be limited to treatment of head & neck cancers.

G. Separate Patentability of Dependent Claims

In the Brief on Appeal, Appellant argued separate patentability for various dependant claims. The Examiner's Answer was silent on this issue. Therefore, the arguments are presented again, below.

Claim 2

Claim 2 is directed to inhibiting the growth of a carcinoma, glioma, sarcoma or melanoma. These tumor types are submitted to be even further removed from the cells of the

Cajot reference, which dealt with lung cancer cell lines growing in culture. As discussed above, Cajot is believed to the only prior art reference considered which in any way concerned p53-positive cells, albeit lung cancer cells *in vitro* and not an actual tumor in a human patient. It is respectfully submitted that a teaching with respect to lung cancer cell lines is in no way predictive of treatment of a "solid tumor" comprised of actual carcinoma, glioma, sarcoma or melanoma tumors in a patient.

Claim 10

Claim 10 is further removed from Cajot as well, in light of the fact that the only animal studies set forth in Cajot are studies involving nude mice. The PTO has consistently taken the position that studies in nude mice, which have no immune system, are inherently unreliable as a predictor of efficacy and utility in humans. Thus, a claim specifically to humans – which are supported by actual human studies – must be accepted as surprising and unexpected vis-à-vis prior art relating only to nude mice.

Claim 12

Claim 12 is directed to at least two administrations of the p53 therapeutic agent to a subject having a p53-positive tumor, followed by tumor resection, and followed by an additional administration of the p53 therapeutic agent. There is simply no prior art that in any way addresses this claim, and certainly no *prima facie* case has even been attempted with respect to this claim.

Claim 17

Claim 17 is directed to continuous perfusion of a natural or artificial body cavity. This is a very particular mode of delivery, and addresses the issue of both tumor type (in a natural body cavity) and post-resection treatment (artificial body cavity). The prior art is totally silent on this aspect of the invention.

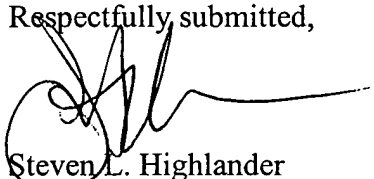
Claims 28, 29 and 31

Claim 28, 29 and 31 claim various aspect of combination therapies with DNA damaging agents, including both radio- and chemotherapies, administration of the DNA damaging agent before, after, or before and after resection. The cited art does not address the use of DNA damaging agents, much less their combination with tumor resection, treatment of microscopic residual disease, or treatment of p53-positive cancers.

V. Conclusion

In light of the preceding, appellant respectfully submits that all of the remaining claims are non-obvious. Therefore, it is respectfully requested that the Board reverse the remaining grounds for rejection.

Respectfully submitted,



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APPENDIX 1: PENDING CLAIMS

1. A method of inhibiting growth of a p53-positive tumor cell in a mammalian subject with a solid tumor comprising the steps of:

(a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and

(b) directly administering said viral expression construct to said tumor *in vivo*, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth,

wherein said tumor comprises cells that express a functional p53 polypeptide.

2. The method of claim 1, wherein said tumor is selected from the group consisting of a carcinoma, a glioma, a sarcoma, and a melanoma.

3. The method of claim 1, wherein said tumor cell is malignant.

4. The method of claim 1, wherein said tumor cell is benign.

5. The method of claim 1, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

6. The method of claim 1, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

7. The method of claim 6, wherein said viral vector is a replication-deficient adenoviral vector.

8. The method of claim 7, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

9. The method of claim 8, wherein said promoter is a CMV IE promoter.

10. The method of claim 1, wherein said subject is a human.

11. The method of claim 7, wherein the expression vector is administered to said tumor at least a second time.

12. The method of claim 11, wherein said tumor is resected following at least a second administration, and an additional administration is effected subsequent to said resection.

13. The method of claim 1, wherein said expression vector is administered in a volume of about 3 ml. to about 10 ml.

14. The method of claim 11, wherein the amount of adenovirus in each administration is between about 10^7 and 10^{12} pfu.

16. The method of claim 1, wherein the expression construct is injected into a natural or artificial body cavity.

17. The method of claim 16, wherein said injection comprises continuous perfusion of said natural or artificial body cavity.

18. The method of claim 16, wherein said contacting is via injection into an artificial body cavity resulting from tumor excision.

19. The method of claim 1, wherein the p53-encoding polynucleotide is tagged so that expression of p53 from said expression vector can be detected.

20. The method of claim 19, wherein the tag is a continuous epitope.

26. The method of claim 1, wherein said tumor is contacted with said expression construct at least twice.

27. The method of claim 26, wherein said multiple injections comprise about 0.1-0.5 ml volumes spaced about 1 cm apart.

28. The method of claim 1, further comprising contacting said tumor with a DNA damaging agent.

29. The method of claim 28, wherein said DNA damaging agent is a radiotherapeutic agent.

30. The method of claim 29, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

31. The method of claim 28, wherein said DNA damaging agent is a chemotherapeutic agent.

32. The method of claim 31, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

36. The method of claim 1, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

37. The method of claim 11, wherein said tumor is contacted with said expression construct at least six times within a two week treatment regimen.

38. (Canceled) A method for inhibiting microscopic residual tumor cell growth in a mammalian subject comprising the steps of:

(a) identifying a mammalian subject having a resectable tumor:
(b) resecting said tumor; and
(c) administering to a tumor bed revealed by resection a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter, the administration resulting in expression of said functional p53 polypeptide in said tumor cells and inhibition of their growth.

39. (Canceled) The method of claim 38, wherein said resectable tumor is a squamous cell carcinoma.

40. (Canceled) The method of claim 38, wherein the endogenous p53 of said resectable tumor is mutated.

41. (Canceled) The method of claim 38, wherein the endogenous p53 of said resectable tumor is wild-type.

42. (Canceled) The method of claim 38, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

43. (Canceled) The method of claim 38, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

44. (Canceled) The method of claim 43, wherein said adenoviral vector is a replication-deficient adenoviral vector.

45. (Canceled) The method of claim 44, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

46. (Canceled) The method of claim 38, wherein said promoter is a CMV IE promoter.

47. (Canceled) The method of claim 38, wherein the resulting tumor bed is contacted with said expression construct at least twice.

48. (Canceled) The method of claim 38, wherein said expression construct is contacted with said tumor bed prior to closing of the incision.

49. (Canceled) The method of claim 44, wherein said the tumor bed is contacted with from about 10^6 to about 10^9 infectious adenoviral particles.

50. (Canceled) The method of claim 47, further comprising contacting said tumor with said expression construct prior to resecting said tumor.

51. (Canceled) The method of claim 50, wherein said tumor is injected with said expression construct.

52. (Canceled) The method of claim 51, wherein said tumor is injected with about 10^6 to about 10^9 infectious adenoviral particles.

53. (Canceled) The method of claim 51, wherein said tumor is injected with a total of about 1 ml to about 10 ml.

54. (Canceled) The method of claim 51, wherein said tumor is injected at least twice.

55. (Canceled) The method of claim 54, wherein each of said injections comprise about 0.1 ml to about 0.5 ml volumes spaced about 1 cm apart.

56. (Canceled) The method of claim 38, wherein the resulting tumor bed is contacted with said expression construct through a catheter.

57. (Canceled) The method of claim 54, wherein said contacting comprises about 10^6 to about 10^9 infectious adenoviral particles.

58. (Canceled) The method of claim 54, wherein said expression construct is contacted with said tumor in total of about 3 ml to about 10 ml.

59. (Canceled) The method of claim 38, wherein the *p53* polynucleotide is tagged so that expression of a p53 polypeptide can be detected.

60. (Canceled) The method of claim 59, wherein the tag is a continuous epitope.

61. (Canceled) The method of claim 38, further comprising contacting said tumor with a DNA damaging agent.

62. (Canceled) The method of claim 61, wherein said DNA damaging agent is contacted before resection.

63. (Canceled) The method of claim 61, wherein said DNA damaging agent is contacted after resection.

64. (Canceled) The method of claim 61, wherein said DNA damaging agent is contacted before and after resection.

65. (Canceled) The method of claim 61, wherein said DNA damaging agent is a radiotherapeutic agent.

66. (Canceled) The method of claim 65, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

67. (Canceled) The method of claim 61, wherein said DNA damaging agent is a chemotherapeutic agent.

68. (Canceled) The method of claim 67, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

73. (Canceled) The method of claim 38, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

74. (Canceled) A method for inhibiting growth of a p53-positive tumor cell in a mammalian subject having a solid tumor comprising the steps of:

(a) surgically revealing said tumor; and
(b) directly administering to said tumor a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter, the administration resulting in expression of said functional p53 polypeptide in said tumor cells and inhibition of their growth.

75. (Canceled) The method of claim 74, wherein said tumor is malignant.

76. (Canceled) The method of claim 74, wherein said tumor is a squamous cell carcinoma.

77. (Canceled) The method of claim 74, wherein said tumor is benign.

80. (Canceled) The method of claim 74, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

81. (Canceled) The method of claim 74, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

82. (Canceled) The method of claim 81, wherein said adenoviral vector is a replication-deficient adenoviral vector.

83. (Canceled) The method of claim 82, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

84. (Canceled) The method of claim 74, wherein said promoter is a CMV IE promoter.

85. (Canceled) The method of claim 74, wherein said tumor is contacted with said expression construct at least twice.

86. (Canceled) The method of claim 74, wherein said expression construct is contacted with said tumor prior to close of the incision.

87. (Canceled) The method of claim 82, wherein said tumor is contacted with from about 10^6 to about 10^9 infectious adenoviral particles.

88. (Canceled) The method of claim 74, wherein said tumor is contacted with said expression construct in a total of about 1 ml to about 10 ml.

89. (Canceled) The method of claim 74, wherein said tumor is injected at least twice.

90. (Canceled) The method of claim 89, wherein each of said injections comprise about 0.1 ml to about 0.5 ml volumes spaced about 1 cm apart.

91. (Canceled) The method of claim 74, wherein said tumor is contacted with said expression construct through a catheter.

92. (Canceled) The method of claim 91, wherein said tumor is contacted with about 10^6 to about 10^9 infectious adenoviral particles.

93. (Canceled) The method of claim 91, wherein said tumor is contacted with an expression construct in a total of about 3 ml to about 10 ml.

94. (Canceled) The method of claim 74, wherein the p53 polynucleotide is tagged so that expression of a *p53* polypeptide can be detected.

95. (Canceled) The method of claim 94, wherein the tag is a continuous epitope.

96. (Canceled) The method of claim 74, further comprising contacting said tumor with a DNA damaging agent.

97. (Canceled) The method of claim 96, wherein said DNA damaging agent is contacted with said tumor before resection.

98. (Canceled) The method of claim 96, wherein said DNA damaging agent is contacted with said tumor after resection.

99. (Canceled) The method of claim 96, wherein DNA damaging agent is contacted with said tumor before and after resection.

100. (Canceled) The method of claim 96, wherein said DNA damaging agent is a radiotherapeutic agent.

101. (Canceled) The method of claim 100, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

102. (Canceled) The method of claim 96, wherein said DNA damaging agent is a chemotherapeutic agent.

103. (Canceled) The method of claim 102, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

108. (Canceled) The method of claim 74, wherein said tumor is located in a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

109. (Canceled) A method of inhibiting tumor cell growth in a mammalian subject having a solid tumor comprising the step of continuously perfusing a tumor site in said patient with a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of their growth.

110. (Canceled) The method of claim 109, wherein said tumor is malignant.

111. (Canceled) The method of claim 109, wherein said tumor is a squamous cell carcinoma.

112. (Canceled) The method of claim 109, wherein said tumor is benign.

113. (Canceled) The method of claim 109, wherein the endogenous p53 of said tumor is mutated.

114. (Canceled) The method of claim 109, wherein the endogenous p53 of said tumor is wild-type.

115. (Canceled) The method of claim 109, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

116. (Canceled) The method of claim 116, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

117. (Canceled) The method of claim 116, wherein said adenoviral vector is a replication-deficient adenoviral vector.

118. (Canceled) The method of claim 117, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

119. (Canceled) The method of claim 109, wherein said promoter is a CMV IE promoter.

120. (Canceled) The method of claim 109, wherein said tumor site is perfused from about one to two hours.

121. (Canceled) The method of claim 109, wherein said subject is a human.

122. (Canceled) The method of claim 109, wherein said tumor site is contacted with said expression vector through a catheter.

123. (Canceled) The method of claim 109, wherein the p53 polynucleotide is tagged so that expression of a *p53* polypeptide can be detected.

124. (Canceled) The method of claim 123, wherein the tag is a continuous epitope.

125. (Canceled) The method of claim 109, further comprising contacting said tumor with a DNA damaging agent.

126. (Canceled) The method of claim 125, wherein said tumor site is contacted with said DNA damaging agent before resection.

127. (Canceled) The method of claim 125, wherein said tumor site is contacted with said DNA damaging agent after resection.

128. (Canceled) The method of claim 125, wherein said tumor site is contacted with said DNA damaging agent before and after resection.

129. (Canceled) The method of claim 125, wherein said DNA damaging agent is a radiotherapeutic agent.

130. (Canceled) The method of claim 129, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

131. (Canceled) The method of claim 125, wherein said DNA damaging agent is a chemotherapeutic agent.

132. (Canceled) The method of claim 131, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

APPENDIX 2: EXHIBITS

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GARY L. CLAYMAN

Serial No.: 08/758,033

Filed: November 27, 1996

For: METHOD AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Group Art Unit: 1632

Examiner: K. Hauda

Atty. Dkt. No.: INRP:041/HYL

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37 C.F.R. 1.8

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4/17/00
DATE

SIGNATURE

AMENDMENT UNDER 37 C.F.R. §1.116

BOX AF

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is in amendment is in partial response to the Final Office Action, mailed on April 12, 1999, and the Examiner's Answer, mailed on February 16, 2000, regarding the above-captioned application. The amendment is filed concurrent with appellants' reply, which is due on April 17, 2000 (April 16th being a Sunday). Please amend the application as follows:

AMENDMENTS

In the Claims:

Please cancel claims 38-68, 73-77, 80-103, 108-132, 137-139, 142 and 143 without prejudice or disclaimer.

REMARKS

This amendment is provided merely to simplify the issues on appeal and in no way represents and acquiescence to the rejections of record. Entry of the amendment is proper as it eliminates grounds for rejection.

Should Examiner Hauda have any questions regarding this amendment, she is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

Steven L. Highlander
Reg. No. 37,642

Date: 4/17/00

Fulbright & Jaworski, LLP
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Austin TX 78701
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SUMMARY OF PENDING CLAIMS

1. A method of inhibiting growth of a p53-positive tumor cell in a mammalian subject with a solid tumor comprising the steps of:

   (a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and

   (b) directly administering said viral expression construct to said tumor *in vivo*, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth,

   wherein said tumor comprises cells that express a functional p53 polypeptide.

2. The method of claim 1, wherein said tumor is selected from the group consisting of a carcinoma, a glioma, a sarcoma, and a melanoma.

3. The method of claim 1, wherein said tumor cell is malignant.

4. The method of claim 1, wherein said tumor cell is benign.

5. The method of claim 1, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

6. The method of claim 1, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

7. The method of claim 6, wherein said viral vector is a replication-deficient adenoviral vector.

8. The method of claim 7, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

9. The method of claim 8, wherein said promoter is a CMV IE promoter.

10. The method of claim 1, wherein said subject is a human.

11. The method of claim 7, wherein the expression vector is administered to said tumor at least a second time.

12. The method of claim 11, wherein said tumor is resected following at least a second administration, and an additional administration is effected subsequent to said resection.

13. The method of claim 1, wherein said expression vector is administered in a volume of about 3 ml. to about 10 ml.

14. The method of claim 11, wherein the amount of adenovirus in each administration is between about 10^7 and 10^{12} pfu.

16. The method of claim 1, wherein the expression construct is injected into a natural or artificial body cavity.

17. The method of claim 16, wherein said injection comprises continuous perfusion of said natural or artificial body cavity.

18. The method of claim 16, wherein said contacting is via injection into an artificial body cavity resulting from tumor excision.

19. The method of claim 1, wherein the p53-encoding polynucleotide is tagged so that expression of p53 from said expression vector can be detected.

20. The method of claim 19, wherein the tag is a continuous epitope.

26. The method of claim 1, wherein said tumor is contacted with said expression construct at least twice.

27. The method of claim 26, wherein said multiple injections comprise about 0.1-0.5 ml volumes spaced about 1 cm apart.

28. The method of claim 1, further comprising contacting said tumor with a DNA damaging agent.

29. The method of claim 28, wherein said DNA damaging agent is a radiotherapeutic agent.

30. The method of claim 29, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

31. The method of claim 28, wherein said DNA damaging agent is a chemotherapeutic agent.

32. The method of claim 31, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

36. The method of claim 1, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

37. The method of claim 11, wherein said tumor is contacted with said expression construct at least six times within a two week treatment regimen.

38. (Canceled) A method for inhibiting microscopic residual tumor cell growth in a mammalian subject comprising the steps of:

(a) identifying a mammalian subject having a resectable tumor:
(b) resecting said tumor; and
(c) administering to a tumor bed revealed by resection a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter, the administration resulting in expression of said functional p53 polypeptide in said tumor cells and inhibition of their growth.

39. (Canceled) The method of claim 38, wherein said resectable tumor is a squamous cell carcinoma.

40. (Canceled) The method of claim 38, wherein the endogenous p53 of said resectable tumor is mutated.

41. (Canceled) The method of claim 38, wherein the endogenous p53 of said resectable tumor is wild-type.

42. (Canceled) The method of claim 38, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

43. (Canceled) The method of claim 38, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

44. (Canceled) The method of claim 43, wherein said adenoviral vector is a replication-deficient adenoviral vector.

45. (Canceled) The method of claim 44, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

46. (Canceled) The method of claim 38, wherein said promoter is a CMV IE promoter.

47. (Canceled) The method of claim 38, wherein the resulting tumor bed is contacted with said expression construct at least twice.

48. (Canceled) The method of claim 38, wherein said expression construct is contacted with said tumor bed prior to closing of the incision.

49. (Canceled) The method of claim 44, wherein said the tumor bed is contacted with from about 10^6 to about 10^9 infectious adenoviral particles.

50. (Canceled) The method of claim 47, further comprising contacting said tumor with said expression construct prior to resecting said tumor.

51. (Canceled) The method of claim 50, wherein said tumor is injected with said expression construct.

52. (Canceled) The method of claim 51, wherein said tumor is injected with about 10^6 to about 10^9 infectious adenoviral particles.

53. (Canceled) The method of claim ~~51, wherein~~ said tumor is injected with a total of about 1 ml to about 10 ml.

54. (Canceled) The method of claim 51, wherein said tumor is injected at least twice.

55. (Canceled) The method of claim 54, wherein each of said injections comprise about 0.1 ml to about 0.5 ml volumes spaced about 1 cm apart.

56. (Canceled) The method of claim 38, wherein the resulting tumor bed is contacted with said expression construct through a catheter.

57. (Canceled) The method of claim 54, wherein said contacting comprises about 10^6 to about 10^9 infectious adenoviral particles.

58. (Canceled) The method of claim 54, wherein said expression construct is contacted with said tumor in total of about 3 ml to about 10 ml.

59. (Canceled) The method of claim 38, wherein the *p53* polynucleotide is tagged so that expression of a p53 polypeptide can be detected.

60. (Canceled) The method of claim 59, wherein the tag is a continuous epitope.

61. (Canceled) The method of claim 38, further comprising contacting said tumor with a DNA damaging agent.

62. (Canceled) The method of claim 61, wherein said DNA damaging agent is contacted before resection.

63. (Canceled) The method of claim 61, wherein said DNA damaging agent is contacted after resection.

64. (Canceled) The method of claim 61, wherein said DNA damaging agent is contacted before and after resection.

65. (Canceled) The method of claim 61, wherein said DNA damaging agent is a radiotherapeutic agent.

66. (Canceled) The method of claim 65, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

67. (Canceled) The method of claim 61, wherein said DNA damaging agent is a chemotherapeutic agent.

68. (Canceled) The method of claim 67, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

73. (Canceled) The method of claim 38, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

74. (Canceled) A method for inhibiting growth of a p53-positive tumor cell in a mammalian subject having a solid tumor comprising the steps of:

(a) surgically revealing said tumor; and
(b) directly administering to said tumor a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter, the administration resulting in expression of said functional p53 polypeptide in said tumor cells and inhibition of their growth.

75. (Canceled) The method of claim 74, wherein said tumor is malignant.

76. (Canceled) The method of claim 74, wherein said tumor is a squamous cell carcinoma.

77. (Canceled) The method of claim 74, wherein said tumor is benign.

80. (Canceled) The method of claim 74, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

81. (Canceled) The method of claim 74, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

82. (Canceled) The method of claim 81, wherein said adenoviral vector is a replication-deficient adenoviral vector.

83. (Canceled) The method of claim 82, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

84. (Canceled) The method of claim 74, wherein said promoter is a CMV IE promoter.

85. (Canceled) The method of claim 74, wherein said tumor is contacted with said expression construct at least twice.

86. (Canceled) The method of claim 74, wherein said expression construct is contacted with said tumor prior to close of the incision.

87. (Canceled) The method of claim 82, wherein said tumor is contacted with from about 10^6 to about 10^9 infectious adenoviral particles.

88. (Canceled) The method of claim 74, wherein said tumor is contacted with said expression construct in a total of about 1 ml to about 10 ml.

89. (Canceled) The method of claim 74, wherein said tumor is injected at least twice.

90. (Canceled) The method of claim 89, wherein each of said injections comprise about 0.1 ml to about 0.5 ml volumes spaced about 1 cm apart.

91. (Canceled) The method of claim 74, wherein said tumor is contacted with said expression construct through a catheter.

92. (Canceled) The method of claim 91, wherein said tumor is contacted with about 10^6 to about 10^9 infectious adenoviral particles.

93. (Canceled) The method of claim 91, wherein said tumor is contacted with an expression construct in a total of about 3 ml to about 10 ml.

94. (Canceled) The method of claim 74, wherein the p53 polynucleotide is tagged so that expression of a *p53* polypeptide can be detected.

95. (Canceled) The method of claim 94, wherein the tag is a continuous epitope.

96. (Canceled) The method of claim 74, further comprising contacting said tumor with a DNA damaging agent.

97. (Canceled) The method of claim 96, wherein said DNA damaging agent is contacted with said tumor before resection.

98. (Canceled) The method of claim 96, wherein said DNA damaging agent is contacted with said tumor after resection.

99. (Canceled) The method of claim 96, wherein DNA damaging agent is contacted with said tumor before and after resection.

100. (Canceled) The method of claim 96, wherein said DNA damaging agent is a radiotherapeutic agent.

101. (Canceled) The method of claim 100, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

102. (Canceled) The method of claim 96, wherein said DNA damaging agent is a chemotherapeutic agent.

103. (Canceled) The method of claim 102, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

108. (Canceled) The method of claim 74, wherein said tumor is located in a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

109. (Canceled) A method of inhibiting tumor cell growth in a mammalian subject having a solid tumor comprising the step of continuously perfusing a tumor site in said patient with a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of their growth.

110. (Canceled) The method of claim 109, wherein said tumor is malignant.

111. (Canceled) The method of claim 109, wherein said tumor is a squamous cell carcinoma.

112. (Canceled) The method of claim 109, wherein said tumor is benign.

113. (Canceled) The method of claim 109, wherein the endogenous p53 of said tumor is mutated.

114. (Canceled) The method of claim 109, wherein the endogenous p53 of said tumor is wild-type.

115. (Canceled) The method of claim 109, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

116. (Canceled) The method of claim 116, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

117. (Canceled) The method of claim 116, wherein said adenoviral vector is a replication-deficient adenoviral vector.

118. (Canceled) The method of claim 117, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

119. (Canceled) The method of claim 109, wherein said promoter is a CMV IE promoter.

120. (Canceled) The method of claim 109, wherein said tumor site is perfused from about one to two hours.

121. (Canceled) The method of claim 109, wherein said subject is a human.

122. (Canceled) The method of claim 109, wherein said tumor site is contacted with said expression vector through a catheter.

123. (Canceled) The method of claim 109, wherein the p53 polynucleotide is tagged so that expression of a *p53* polypeptide can be detected.

124. (Canceled) The method of claim 123, wherein the tag is a continuous epitope.

125. (Canceled) The method of claim 109, further comprising contacting said tumor with a DNA damaging agent.

126. (Canceled) The method of claim 125, wherein said tumor site is contacted with said DNA damaging agent before resection.

127. (Canceled) The method of claim 125, wherein said tumor site is contacted with said DNA damaging agent after resection.

128. (Canceled) The method of claim 125, wherein said tumor site is contacted with said DNA damaging agent before and after resection.

129. (Canceled) The method of claim 125, wherein said DNA damaging agent is a radiotherapeutic agent.

130. (Canceled) The method of claim 129, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

131. (Canceled) The method of claim 125, wherein said DNA damaging agent is a chemotherapeutic agent.

132. (Canceled) The method of claim 131, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

137. (Canceled) The method of claim 109, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

138. (Canceled) The method of claim 1, wherein said expression vector is administered topically.

139. (Canceled) The method of claim 1, wherein said expression vector is administered intratumorally.

142. (Canceled) The method of claim 74, wherein said expression vector is administered topically.

143. (Canceled) The method of claim 74, wherein said expression vector is administered intratumorally.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GARY L. CLAYMAN

Serial No.: 08/758,033

Filed: November 27, 1996

For: METHOD AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Group Art Unit: 1632

Examiner: K. Hauda

Atty. Dkt. No.: INRP:041/HYL

CERTIFICATE OF MAILING
37 C.F.R. 1.8

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DATE

SIGNATURE

DECLARATION OF DR. GARY L. CLAYMAN UNDER 37 C.F.R. § 1.131

Hon. Assistant Commissioner for Patents
Washington, D.C. 20231

I, Gary L. Clayman, D.D.S., M.D., declare that:

1. I am a U.S. citizen residing at 6353 Westchester Street, Houston, Texas. I am Associate Professor of Surgery and Deputy Chairman of the Department of Head and Neck Surgery at the University of Texas M.D. Anderson Cancer Center. A copy of my curriculum vitae outlining my education and research training is attached (Exhibit A).

2. I am the inventor of the above-captioned application and a portion of my research has been sponsored by Introgen Therapeutics, Inc., a company that has licensed this technology.

3. I am a co-author of Clayman *et al.*, published in *Cancer Research* (Exhibit B), along with Drs. Adel K. El-Naggar, Jack A. Roth, Wei-Wei Zhang, Helmuth Goepfert, Dorothy L. Taylor, and Ta-Jen Liu. I also am co-author of Liu *et al.*, published in *Cancer Research* (Exhibit C), along with Drs. Liu, El-Naggar, Taylor, Timothy J. McDonnell, Kim D. Steck, and Mary Wang.

4. Drs. El-Naggar, Roth, Zhang, Goepfert, Taylor, Liu, McDonnell, Steck and Wang, the non-inventor co-authors of this paper, did not contribute to the conception of the present invention of using Adp53 for the treatment of head and neck cancer. Each of these individuals acted under the supervision and direction of myself in generating the results reported in these papers, or as a reviewer of the manuscripts prior to publication.

5. Dr. Adel K. El-Naggar performed the pathologic analysis in Exhibit B and the fluorescent analysis in Exhibit C.

6. Dr. Jack A. Roth provided the Adp53 vector in Exhibits B and C.

7. Dr. Wei-Wei Zhang developed the Adp53 vector in Exhibit B.

8. Dr. Helmuth Goepfert reviewed the Exhibit B manuscript prior to publication.

9. Dr. Dorothy L. Taylor developed and maintained the head and neck cancer cell lines, and assisted in the experiments described in Exhibits B and C.

10. Dr. Ta-Jen Liu performed the *in vitro* propagation of the Adp53 vector in Exhibit B and performed the DNA fragmentation analysis in Exhibit C.

11. Dr. Timothy J. McDonnell reviewed the Exhibit C manuscript prior to publication.

12. Dr. Kim D. Steck performed the technical fluorescent sorting studies in Exhibit C.

13. Dr. Mary Wang performed infection assays and viral propagation in Exhibit C.

14. I hereby declare that all statements made herein of my knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the U.S. Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6/18/98
Date

Gary L. Clayman, D.D.S., M.D.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GARY L. CLAYMAN

Serial No.: 08/758,033

Filed: November 27, 1996

For: METHOD AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Group Art Unit: 1632

Examiner: K. Hauda

Atty. Dkt. No.: INRP:041/HYL

CERTIFICATE OF MAILING
37 C.F.R. 1.8

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9/13/99
DATE

SIGNATURE

DECLARATION OF DR. JAMES A. MERRITT

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

I, James A. Merritt, declare that:

1. I am the Vice President of Clinical Affairs at Introgen Therapeutics, Inc., ("Introgen"), licensee of the above-captioned application, and have held this position for three years. I am an internist and board certified medical oncologist.

2. In this capacity, I regularly deal with cancer therapy clinical trials involving the use of p53 gene therapy. As such, I am familiar with the results from both Phase I and Phase II clinical studies being carried out by Introgen.

3. In the Phase I study reported in Clayman *et al.*, *J. Clin. Oncol.* 16:221-2232 (1998) (Exhibit 1), thirty-three patients with recurrent head & neck cancer were treated with intratumoral injections of Ad-p53. The treatment regimen consisted of at least one course of Ad-p53 (three times a week for two weeks). Of these, eighteen patients had non-resectable tumors (and received multiple treatment courses with two week rest between courses; continuing until disease progression or withdrawal of consent), permitting post-treatment assessment of tumor progression. Of these eighteen, twelve were p53+ by sequencing of tumor cell DNA. Of these twelve, two patients had greater than 50% tumor regression, four had stable disease, five had progressive disease, and in one the outcome of treatment could not be evaluated. By comparison, of the remaining six non-resectable patients, one was non-evaluable for p53 status. Of the five patients with mutated p53 genes, four exhibited progressive disease while two exhibited stable disease. (Of the total of 33 patients entered in the study, the remaining 15 underwent complete resection of their tumor three days after a single course of treatment, and could not be rigorously assessed for clinical response.)

4. In a subsequent Phase II trial (studies designated T201 and T202, analyzed February 26, 1999), 154 patients with recurrent, non-resectable head & neck

tumors were enrolled for Ad-p53 treatment on either (a) three consecutive (1, 2, 3), or (b) six biconsecutive (1, 3, 5, 8, 10, 12) days, with treatment cycles repeated every four weeks. One hundred forty-seven patients were treated, with 124 being evaluable. Of this latter group, two patients showed a complete response, two were partially responsive, and 24 exhibited stable disease for a period of three to seven months.

p53 status was ascertainable in 71 of these patients. Comparing clinical response to treatment on the basis of p53+ and p53- status (as determined by DNA sequencing), the following results were obtained:

SUMMARY OF PHASE II CLINICAL DATA BY p53 STATUS (n=71)

p53 status*	CR	PR	SD	PD	NE
p53+	0	2	6	21	3
p53-	0	1	6	27	5

* as determined by sequencing of exons 1-10; some patients had unknown p53 status.
CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease; NE – non-evaluable

5. These results demonstrate that treatment of p53+ tumors using a p53-expressing vector is at least as clinically useful as treatment of tumors that lack a wild-type p53 molecule.

6. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this patent, and any reexamination certificate issuing thereon.

25 August 1999
Date

Dr. James A. Merritt

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
GARY L. CLAYMAN

Serial No.: 08/758,033

Filed: November 27, 1996

For: METHOD AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER

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DECLARATION UNDER 37 C.F.R. §1.131 OF DR. GARY CLAYMAN

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

I, Gary Clayman, declare that:

1. I am a U.S. citizen residing at 6353 Westchester St., Houston, Texas. I am Associate Professor of Surgery and Deputy Chairman of the Department of Head and Neck Surgery at the University of Texas M.D. Anderson Cancer Center.

2. I am the Gary Clayman named as an inventor of the above-captioned application. A portion of my research has been sponsored by Introgen Therapeutics, Inc., the exclusive licensee of this application.

3. In January of 1995, I published a paper (Liu *et al.*, *Cancer Res.* 55:1-6 (1995)) that reported the Ad-p53 infection of cell lines with both mutated and wild-type p53. A similar report was in July of the same year (Clayman *et al.*, *Cancer Res.* 55:3117-3122 (1995)). I understand that the Examiner in charge of examining the referenced application has previously taken the position that these papers teach the use of adeno-p53 in the therapy of tumors *in vivo*, including the therapy of p53-positive tumors (Office Action of 2/17/99).

4. The studies set forth in these papers demonstrate that I had achieved the subject matter that they disclose in the United States at least as of their date of publication, the earliest publication date as between the two being January, 1995).

5. I understand that the papers of Katayose *et al.*, *Clin. Cancer Res.* 1:889-897 (1995) and Srivastava *et al.*, *Urology* 46:843-848 (1995), both published after January, 1995, are being cited against various claims of the present application for their alleged teaching of *in vitro* studies using a p53-expressing adenovirus to infect tumor cells. Based on the earlier publication of my two articles referenced above, it is clear that I had in my possession at least equivalent, and indeed more extensive, data than is taught in the Katayose and Srivastava references at a time prior to their respective publication dates.

6. I declare that all statements made herein of myr own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this patent, and any reexamination certificate issuing thereon.

11/8/99
Date

Dr. Gary Clayman

1 : *In Vivo* 1992 Nov-Dec;6(6):605-10 Related Articles, Books

Related Resources

Differential effects of butyrate derivatives on human breast cancer cells grown as organotypic nodules in vitro and as xenografts in vivo.

Planchon P, Magnien V, Beaupain R, Mainguene C, Ronco G, Villa P, Brouty-Boye D

Institut d'Oncologie Cellulaire et Moleculaire Humaine, Hopital Avicenne, Bobigny, France.

The antiproliferative and cytodifferentiating effects of a new stable butyric derivative, monobut-3, were compared using human MDA-MB-231 breast cancer cells grown in three dimension as either in vitro tumor nodules or in vivo xenograft tumors. In in vitro tumor nodules, monobut-3 exhibited marked growth inhibitory effects consistent with the results obtained in monolayer cell cultures. Some functional cell differentiation was also detected in treated nodules. In in vivo xenografts, monobut-3 significantly decreased MDA-MB-231 tumor take but did not affect the rate of tumor growth. No difference was noted in the histological characteristics of the xenografts between untreated and treated mice. Moreover, once monobut-3 treatment was discontinued, tumor growth rapidly resumed in tumor-free animals. The decreased efficacy of monobut-3 in in vivo MDA-MB-231 xenografts as compared to in vitro tumor nodules indicates that factors related to host environment may still limit the clinical effectiveness of this compound.

PMID: 1296809, UI: 93208321

Pharmacodynamics of cisplatin in human head and neck cancer: correlation between platinum content, DNA adduct levels and drug sensitivity in vitro and in vivo.

Welters MJ, Fichtinger-Schepman AM, Baan RA, Jacobs-Bergmans AJ, Kegel A, van der Vijgh WJ, Braakhuis BJ

Toxicology Division, TNO Nutrition and Food Research Institute, Zeist, The Netherlands.

Total platinum contents and cisplatin-DNA adduct levels were determined in vivo in xenografted tumour tissues in mice and in vitro in cultured tumour cells of head and neck squamous cell carcinoma (HNSCC), and correlated with sensitivity to cisplatin. In vivo, a panel of five HNSCC tumour lines growing as xenografts in nude mice was used. In vitro, the panel consisted of five HNSCC cell lines, of which four had an in vivo equivalent. Sensitivity to cisplatin varied three- to sevenfold among cell lines and tumours respectively. However, the ranking of the sensitivities of the tumour lines (in vivo), also after reinjection of the cultured tumour cells, did not coincide with that of the corresponding cell lines, which showed that cell culture systems are not representative for the in vivo situation. Both in vitro and in vivo, however, significant correlations were found between total platinum levels, measured by atomic absorption spectrophotometry (AAS), and tumour response to cisplatin therapy at all time points tested. The levels of the two major cisplatin-DNA adduct types were determined by a recently developed and improved 32P post-labelling assay at various time points after cisplatin treatment. Evidence is presented that the platinum-AG adduct, in which platinum is bound to guanine and an adjacent adenine, may be the cytotoxic lesion because a significant correlation was found between the platinum-AG levels and the sensitivities in our panel of HNSCC, in vitro as well as in vivo. This correlation with the platinum-AG levels was established at 1 h (in vitro) and 3 h (in vivo) after the start of the cisplatin treatment, which emphasizes the importance of early sampling.

PMID: 10408697, UI: 99335182

Revised: April 3, 2000.

Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma in vitro and in vivo.

Vingerhoeds MH, Steerenberg PA, Hendriks JJ, Dekker LC, Van Hoesel QG, Crommelin DJ, Storm G

Related Resources

Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, The Netherlands.

This paper deals with the utility of immunoliposomes for the delivery of doxorubicin (DXR) to human ovarian carcinoma cells in vitro and in vivo. We aimed to investigate whether immunoliposome-mediated targeting of DXR to ovarian cancer cells translates in an enhanced anti-tumour effect compared with that of non-targeted DXR liposomes (lacking the specific antibody). Target cell binding and anti-tumour activity of DXR immunoliposomes were studied in vitro and in vivo (xenograft model of ovarian carcinoma). In vitro we observed that target cell binding and cell growth inhibition of DXR immunoliposomes is superior to that of non-targeted DXR-liposomes. However, in vivo, despite the efficient target cell binding and good anti-tumour response of DXR-immunoliposomes, no difference in anti-tumour effect, compared with non-targeted DXR-liposomes, could be determined. The results indicate that premature DXR leakage from immunoliposomes occurring before the actual target cell binding and subsequent DXR association with the tumour cells, explains why no significant differences in anti-tumour activity between DXR-immunoliposomes and non-targeted DXR-liposomes were observed in vivo.

PMID: 8855969, UI: 97008852

1 : *In Vivo* 1996 May-Jun;10(3):329-33 Related Articles, Books, LinkOut

The effect of high dose vitamin A on the morphology and proliferative activity of xenograft lung and head and neck cancer.

Mourad WA, Bruner JM, Vallieres E, McName C, Alabdulwahed S, Scott K, Oldring DJ

Department of Pathology, University of Alberta, Edmonton, Canada.

In vitro studies have suggested that vitamin A lowers invasive potential of squamous cell carcinoma. Epidemiological data have also indicated that high dose vitamin A may improve survival in patients with previously resected lung carcinoma. To our knowledge, no studies have attempted to test the in vivo effect of vitamin A on the morphology and growth rate of lung and head and neck cancer. Freshly resected tumor cell suspensions were obtained by ex vivo fine needle aspiration and injected subcutaneously in duplicate in athymic male nude mice. Two to six weeks post-engraftment tests and controls were separated for each xenograft. Mice with test xenografts were given water soluble vitamin A (Aquasol ATM, Astra pharmaceutical, Westborough, MA, U.S.A) at a dose of 10,000 U/Kg/day intraperitoneally for 6 to 10 weeks (median 8 weeks). One to two hours prior to sacrifice bromodexouridine (BrdU) was injected intraperitoneally to assess the S-phase fraction in both test and control xenografts. Blood vitamin A levels in test and control animals were measured after sacrifice using high performance liquid chromatography (HPLC). Sections of test and control xenografts were routinely stained to assess morphologic differentiation and mitotic counts. Unstained sections of xenografts were immunostained by the antibody to BrdU to test for BrdU labeling index (BLI) reflecting S-phase fraction (SPF) and also by the MIB-1 antibody to assess proliferative activity. Eighteen tumors were studied. These included 9 squamous cell carcinomas of the lung, 5 squamous cell carcinomas of the head and neck, and 4 adenocarcinomas of the lung. Blood levels of vitamin A in test animals were 7 to 23 times those of the control animals (median 13 times). Neovascularization of the xenografts was seen in all cases. The morphology and mitotic activity of the test and control xenografts showed no significant difference. SPF and proliferative activity measured by BrdU and MIB-1 immunolabelling respectively showed no significant difference between test and control xenografts. Our study suggests that there is no significant in vivo effect of high dose vitamin A on the morphology and growth rate of xenografted non small cell carcinoma of the lung or squamous cell carcinoma of the head and neck.

Transforming growth factor-beta and response to anticancer therapies in human liver and gastric tumors in vitro and in vivo.

Liu P, Menon K, Alvarez E, Lu K, Teicher BA

Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA.

Liver cancer and gastric cancer are the most common solid tumors worldwide. Transforming growth factor-beta (TGF-beta) production and lack of response to TGF-beta growth inhibitory effects have been associated with tumor progression and therapeutic resistance. HepG2, Hep3B, and SK-HEP-1 human liver cancer lines produce 3, 5.7, and 2.5 ng TGF-beta1; 1.4, 2, and 4 ng TGF-beta2 and 0.15, 0.2 and 0.22 ng TGF-beta3 per 107 cells (24 h). Expression of the TGF-beta type I receptor is 20x, 1x, and 0.6x the level in mink lung MvLu1 cells in the HepG2, Hep3B, and SK-HEP-1 cells, respectively. HepG2 and Hep3B cells do not express the TGF-beta type II receptor while SK-HEP-1 cells express 7x the level found in mink lung MvLu1 cells. Hs 746T, KATO III, RF-1, and RF-48 human gastric cancer cell lines produce 12. 5, 0.35, 0.4, and 0.4 ng TGF-beta1; 2.6, 0.95, 0.5, and 0.52 ng TGF-beta2 and 0.42, 0.17, 0.12, and 0.14 ng TGF-beta3 per 107 cells (24 h). Expression of TGF-beta type I receptor is 0.7x, 0.7x, 0.8x, 0.6x the level in mink lung MvLu1 cells in the Hs 746T, KATO III, RF-1 and RF-48 cells, respectively. KATO III cells are lacking in the TGF-beta type II receptor while Hs 746T, RF-1 and RF-48 cells express 10x, 0.8x, and 1x the levels in mink lung MvLu1 cells. The IC50 for TGF-beta1 is >>10 ng/ml in all of these lines except RF-48 where TGF-beta1 is mitogenic. The response of the cell lines to radiation, doxorubicin, mitomycin C, cisplatin, 5-fluorouracil, methotrexate, and gemcitabine showed that SK-HEP-1 was the most drug resistant liver cancer cell line and KATO III was the most drug resistant gastric cancer cell line. Overall, there was no correlation between TGF-beta secretion in cell culture and sensitivity of the cells to anticancer agents. Increased TGF-beta1 levels were detectable in the plasma of nude mice bearing Hep3B and Hs 746T xenografts. Those tumors which secreted greater amounts of TGF-beta were more therapeutically resistant in vivo.

PMID: 10675495, UI: 20141554

Tumour-growth suppression in nude mice by a murine monoclonal antibody: factors hampering successful therapy.

Johansson C, Segren S, Lindholm L

Pharmacia CanAg, Goteborg, Sweden.

The murine MAb C215 has been shown to mediate ADMMC in vitro and to have a tumour-growth-suppressive effect on xenografted COLO 205 human colocarcinoma cells in nude mice. To overcome the limitations of MAb therapy, it is necessary to understand the underlying mechanisms of tumour-growth suppression. In the present work, we have used C215 to define the importance of different parameters involved in tumour therapy with murine IgG2a antibodies. The results show that there exists a period of roughly 2 days after inoculation into animals during which the tumour cells are sensitive to an inhibitory antibody-mediated effect. After this initial period, the in-vivo sensitivity of tumour cells to antibody-mediated inhibition is much reduced. Tumour cells can remain "dormant" and, despite ongoing antibody treatment, develop into tumours with a reduced growth rate, which is not caused by outgrowth of antigen-deficient tumour cells. Finally, a pronounced dependence of antibody-mediated tumour suppression on antibody dose was observed.

PMID: 1708368, UI: 91210009

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: GARY L. CLAYMAN

Serial No.: 08/758,033

Filed: November 27, 1996

For: METHODS AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Group Art Unit: 1632

Examiner: K. Hauda

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DECLARATION OF DR. LOUIS ZUMSTEIN UNDER 37 C.F.R. 1.132

BOX AF
Assistant Comissioner of Patents
Washington, D.C. 20231

I, Louis Zumstein, Ph.D., declare that:

1. I am the Director of Research at Introgen Therapeutics, Inc. ("Introgen"), licensee of the above-captioned application. I am a citizen of the United States of America and I reside at 1912 Vermont St., Houston, Texas, USA.

The Studies Shown by Cajot et al. are Not Valid In Vivo Studies

2. I understand that the PTO examiner in charge of the referenced case has taken the position that the Cajot *et al.* reference discloses *in vivo* studies involving the use of p53 gene therapy in wild-type p53 expressing tumors. This is not the case. The nude mouse studies described in Cajot *et al.* were not true *in vivo* studies. The lung tumor cell lines at issue there were transfected with the p53 vector *ex vivo* (*in vitro)* and then injected into the nude mice only AFTER *in vitro* treatment. Such an assay is not a true *in vivo* assay since one is not establishing the tumor *in vivo* first, and then treating the established tumor.

3. Where one employs an *ex vivo* assay such as was employed by Cajot *et al.*, there is no test for the effects of the therapy on the tumor *in situ* in the patient's body -- *e.g.*, is the vector capable of penetrating and entering the tumor cells *in situ*, does the therapy have an effect on the tumor mass when the tumor mass in actively growing in an animal (as opposed to mere cells in a test tube), is there sufficient distribution of the vector to cells of the tumor, and sufficient expression within those cells, to effect a noticeable growth inhibitory effect, can the material pass through the extracellular matrix that comprises the tumor mass, are there extracellular components in the tumor milieu that might block uptake, *etc.* Studying the effect of a gene such as the p53 gene on cells *in vitro* tells one little about the ability of a gene to work as a tumor suppressor gene in vivo, and would not be relevant to the claims at issue in this appeal, which I understand are directed to direct administration to a tumor *in vivo*.

4. I am familiar with the proper way to carry out a nude mouse assay where one desires to more closely reflect an *in vivo* anticancer therapy. An example is set forth in the

article of Ueyama, "Utilization of Nude Mice in Research on Human Cancer," in Animal Models: Assessing the Scope of Their Use in Biomedical Research, 1987 (Exhibit BB). Ueyama extols the importance of using the nude mouse assay in assessing anticancer agents, and points out that the assay involves first establishing the cancer in the nude mouse by xenotransplantation and then treating the resultant tumor with the anticancer agent *in situ* (p. 289). As with gene therapy, it would make little sense to treat the cancer cells in a test tube with the anticancer drug and then transplant the cells into the mouse if one desires to test the effect of the drug on the tumor in vivo.

The One Study Performed by Cajot in Nude Mice Supports the Conclusion that p53 Therapy WOULD NOT be Effective Against Wild-Type p53 Tumors

5. The one study reported by Cajot *et al.* in nude mice, albeit using *ex vivo* rather than *in vivo* therapy as pointed out above, would not support a conclusion that p53 would be an effective therapy against wild-type p53 tumors. In contrast, the Cajot *et al.* study would argue against such a conclusion. In the nude mouse study shown in Cajot's Figure 3, the only transfectant purported to be "wild type" was the cell line designated "X833.W2." However, it is clear from Figure 1C and from the text that "X833.W2" is not a wild type clone at all -- it expressed a mutant p53 ("X833.W2 was shown by Western blot analysis to express what appears to be a mildly truncated form of the p53 protein", p. 6958, col. 2). Furthermore, there are 17 other allegedly wild-type p53 transfectants reported (see Figure 1A). Inexplicably, no nude mouse growth results are shown with any other wild type p53 transfectant. Yet, the text explicitly discloses that such growth assays were conducted with at least 4 other wild-type p53 transfectants but that these transfectants were not growth inhibited -- that these wild type transfectants were failures (see p. 6958, col. 1, last full sentence, and middle of col. 2).

6. Thus, from the studies reported in Cajot *et al.*, it is evident that out of at least 18 different wild-type p53 clones that were analyzed, only one was reported to exhibit some form of growth suppression -- the other 17 were not reported to show any growth suppression. Yet, this one clone that was allegedly growth suppressed, X833.W2, in fact expressed only a mutant p53. The only reasonable scientific conclusion that one can draw from these studies is that the introduction of the p53 gene into the wild-type p53 lung cancer cell line was not reported to be effective in reducing the growth rate of the lung cancer cells in 17 of the 18 purported "wild type" transfectants. In the one instance where there did appear to be growth suppression, such suppression could not be attributed to wild type p53 expression.

There Is No Expectation from Purely In Vitro Cancer Treatment Results that a Particular Therapy Will Be Efficacious In Vivo

7. There is no expectation from purely in vitro cancer treatment results involving cell lines, that such a treatment will be efficacious in vivo. The scientific literature is indeed replete with examples of cancer treatments that showed promise *in vitro* only to fail *in vivo*. For example, Planchon *et al.* (1992) showed that butyrate derivatives inhibited growth of breast cancer cell monolayers *in vitro*, but failed to affect the rate of tumor growth *in vivo*. Exhibit U. Welters *et al.* (1999), in examining the effects on cisplatin in head & neck cancers, found a lack of correlation between studies on *in vitro* tumor cell lines and *in vivo* tumors. Exhibit V. Vingerhoeds *et al.* (1996) similarly compared the effects of doxorubicin on ovarian carcinoma cells and found that *in vitro* inhibition was not observed *in vivo*. Exhibit W. Mourad *et al.* (1996) showed that high doses of vitamin A inhibited head & neck and lung cancers *in vitro*, but had no similar effects *in vivo*. Exhibit X. Liu *et al.* (2000) disclosed that, *in vivo*, secretion of

TGF-□ correlated with resistance to tumor therapy, while no correlation was observed *in vitro.* Exhibit Y. Finally, Johansson *et al.* (1991) demonstrated that a murine monoclonal antibody inhibited cancer cells *in vitro*, but that *in vivo* inhibition was limited to administration two days after inoculation into animals, hardly a relevant clinical situation. Exhibit Z. Accordingly, there is no reasonable expectation that a cancer therapy will prove to be efficacious in vivo based on purely *in vitro* studies.

Pre-Clinical In Vivo Studies with Adenoviral p53 in Wild Type p53 Tumors Other than SCCHN

8. In my capacity as Director of Research at Introgen, I supervised the pre-clinical evaluation of an adenoviral vector that expresses wild type p53 and thus am generally familiar with pre-clinical studies utilizing this vector that were conducted by Introgen and its collaborators. The adenoviral vector used in these pre-clinical evaluations, referred to as "Ad-p53," was constructed by removing the E1 portion of the adenoviral genome, and replacing this with the p53 gene under the control of a CMV promoter ("Ad-p53"). This construct was thus prepared in a manner consistent with the description of adenoviral vectors as gene transfer vectors in the referenced specification on pages 24 to 26 and page 45 lines 14 to 23.

9. I also understand that the PTO examiner has taken the position that even if the SSCHN clinical studies are statistically significant, they are only relevant with respect to head and neck cancer and are not indicative of efficacy with respect to other wild type p53 expressing cancers. While no other clinical studies in human cancer patients having other types of cancers have been scored to date, we, or our collaborators, have conducted studies involving other wild type p53 expressing cancers in appropriate nude mouse assays. These studies involved the

treatment of established *in vivo* tumors, rather than treatment of cell lines *in vitro*. In each case, these studies have demonstrated that p53 therapy against wild type tumors has been surprisingly efficacious.

10. In one study, involving an *in vivo* model of adenoviral p53 therapy of prostate tumors, the wild type p53 prostate cancer cell line LNCap was used to establish tumors in the prostates of nude mice. Once established, the prostate tumors were treated with intra-prostatic injection of adenoviral p53. Since it is difficult to measure tumor volumes in this model, and since LNCap cells produce PSA, serum PSA levels, an accepted surrogate for prostate tumor volume, were measured. Adenoviral p53 treatment of this p53 wild-type tumor caused significant reduction of PSA levels, evidencing a reduction in the growth of these p53 wild-type tumors. The actual data is shown in attached Figure 1.

11. In another study, involving an *in vivo* model of adenoviral p53 therapy of lung tumors, the wild type p53 lung cancer cell line A549 was used to establish subcutaneous tumors in nude mice. Once established, tumors were treated with intra-tumoral injection of adenoviral p53. Adenoviral p53 injection into this p53 wild-type tumor caused a significant delay in the rate of tumor growth. The data is shown in attached Figure 2.

12. In yet another study, involving an *in vivo* model of adenoviral p53 therapy of cervical tumors, the wild type p53 cervical cancer cell lines SiHa and MS751 were used to establish subcutaneous tumors in nude mice. Once established, tumors were treated with intra-tumoral injection of adenoviral p53. Adenoviral p53 injection into both of these p53 wild-type

tumors caused a large and significant delay in the rate of tumor growth. The data is shown in attached Figures 3A (single Ad-p53 injection 25 d after tumor cell implantation), 3B (3 injections of Ad-p53 at 25 d post implantation) and 3C (6 injections of Ad-p53 post implantation).

13. In conclusion, it is evident that p53 gene therapy is highly active in the therapy of a number of wild type p53 expressing tumors other than SCCHN, as indicated in an *in vivo* nude mouse system that we have found to be trustworthy. This is directly contrary to the general expectation in the art, prior to the Clayman *et al.* discovery, that p53 therapy would not prove to be clinically effective against tumors that express wild type p53.

14. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or

imprisonment, or both, under § 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this patent, and any reexamination certificate issuing thereon.

April 17, 2000
Date

Dr. Lou Zumstein

Ad-p53 Treatment Leads to a Decrease in PSA Values in Orthotopic Models of $p53^{wild\text{-}type}$ Human Prostate Cancer

Since LNCaP cells produce PSA, the orthoptic model used for these studies closely parallels human prostate cancer. Serum PSA obtained through tail vein bleeding is a surrogate for tumor weight and/or volume.

		Serum PSA in ng/ml (Mean ± S.E.)	
Group	N	Pre-treatment	Post-treatment
PBS alone	5	2.82 ± 0.52	44.74 ± 8.44
AdCMV-pA	10	5.52 ± 0.69	25.83 ± 6.78
Ad-p53	11	4.62 ± 1.10	12.18 ± 4.24

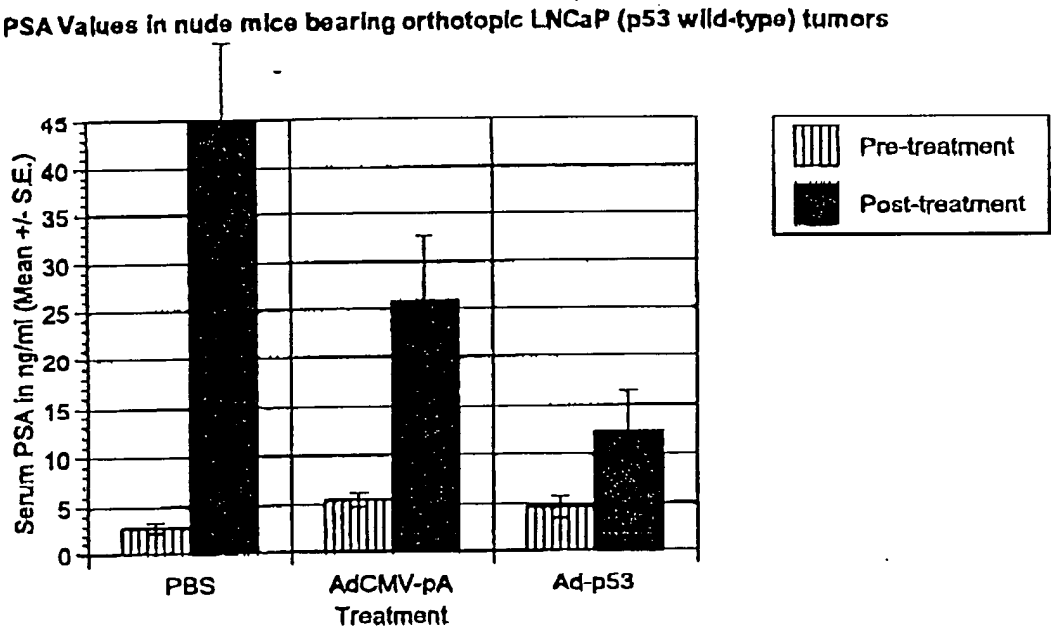

FIG. 1

Effect of Ad5CMV-*p53* ± 5 Gy irradiation on the growth of A549 ($p53^{wild-type}$) subcutaneous xenografts. Established tumors were treated after reaching a size of ~200 mm^3. Treatment was every other day for 3 injections of PBS or vector (total dose 9 x 10^9 pfu). Tumors were treated with 5 Gy on Day 6. Closed square: PBS alone; open square: PBS + 5 Gy; closed triangle: Ad-luc alone; open triangle: Ad-luc + 5 Gy; closed round: Ad-p53 alone; open round: Ad-p53 + 5 Gy.

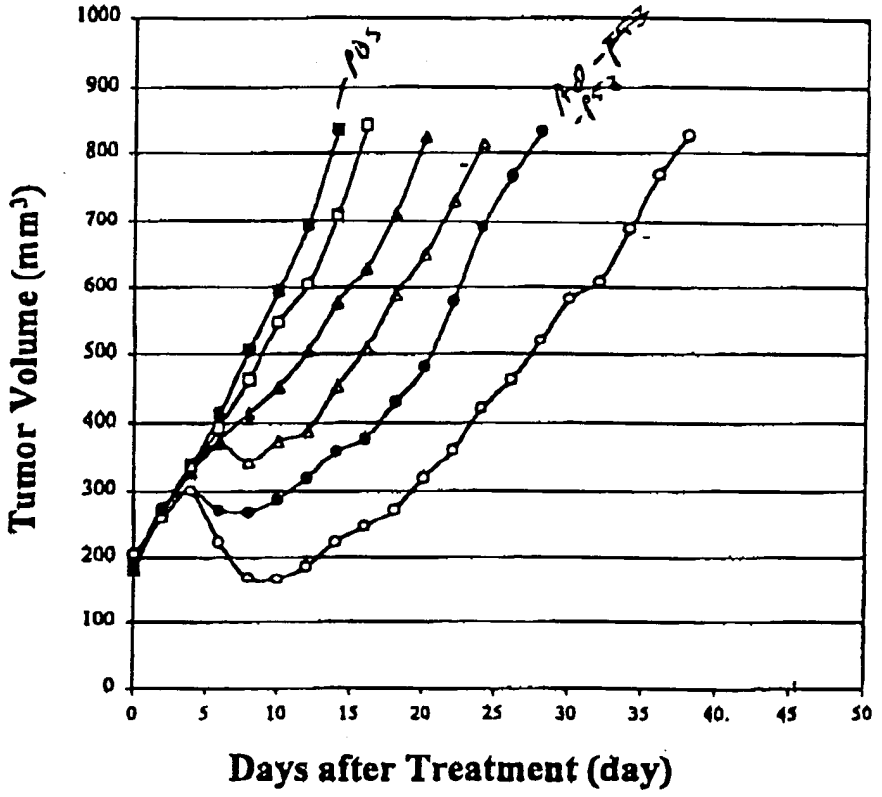

FIG. 2

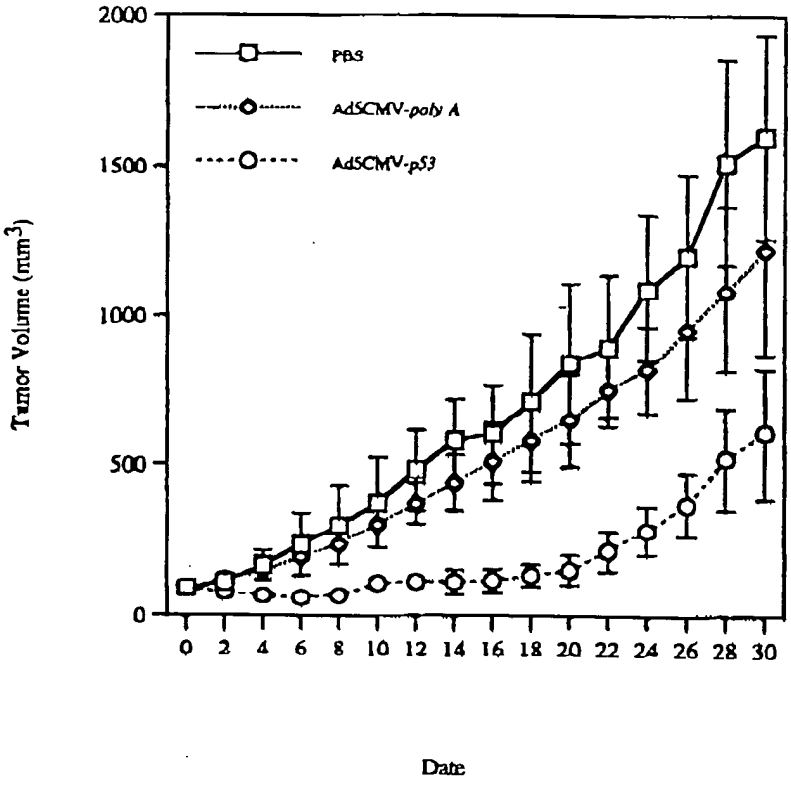
2000
1500
1000
500
0
Tumor Volume (mm3)
PBS
AdSCMV-poly A
AdSCMV-p53
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Date

FIG. 3A

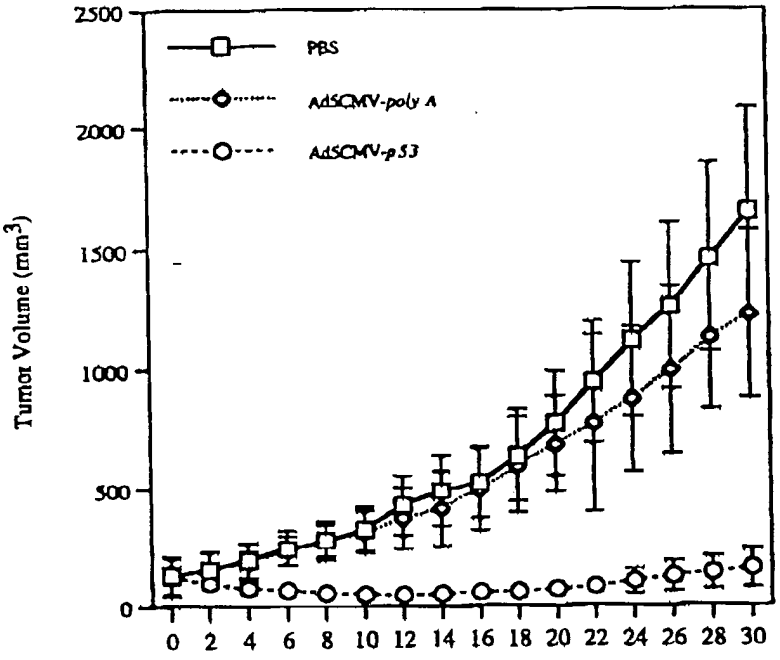
2500
2000
1500
1000
500
0
PBS
AdSCMV-poly A
AdSCMV-p53
Tumor Volume (mm3)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Days

FIG. 3B

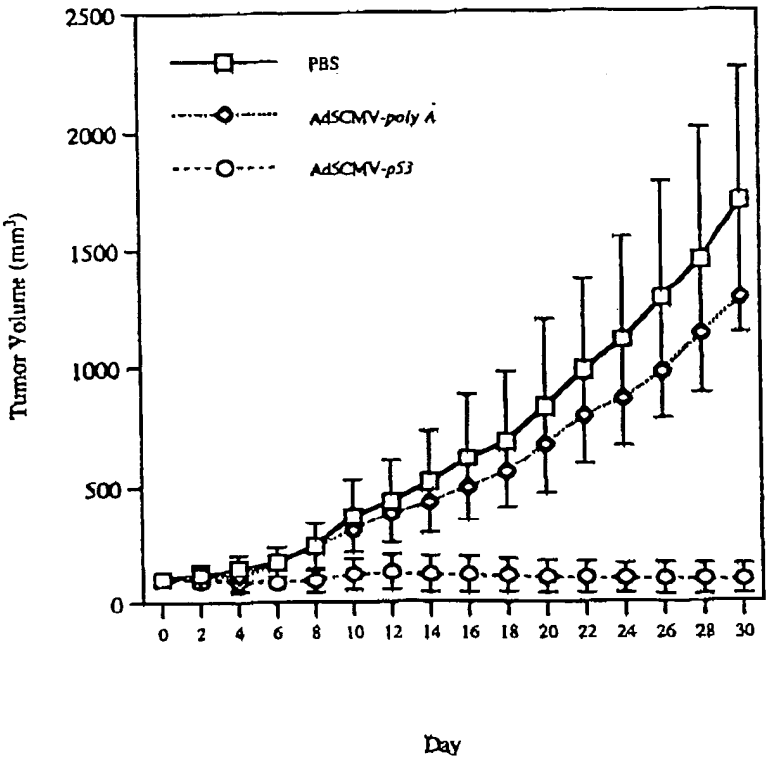
2500
2000
1500
1000
500
0
Tumor Volume (mm³)
PBS
AdSCMV-poly A
AdSCMV-p53
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Day

FIG. 3C

ANIMAL MODELS: Assessing the Scope of Their Use in Biomedical Research

Proceedings of the Sixth Charles River International Symposium on Laboratory Animals, held in Kyoto, Japan, October 8–9, 1985

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UTILIZATION OF NUDE MICE IN RESEARCH ON HUMAN CANCER - SCREENING SYSTEM OF ANTICANCER AGENTS USING HUMAN TUMOR XENOGRAFTS IN NUDE MICE

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ABSTRACT

Human cancers including eleven lines of stomach cancer, thirteen lines of lung cancer and six lines of glioma xenotransplated in nude mice were tested for experimental treatment by seven different anticancer agents in the present project. The doses used in this experiment were the maximum tolerated dose (MTD) and rational dose (RD) which was determined to maintain the blood level of drugs in nude mice the same as that in humans to obtain clinical effectiveness.

In this experimental regimen, the response rate (number of effective lines/number of total lines tested) in the MTD treated group was much higher than the clinical response rate, but the response rate in the RD-administered group was almost the same as the clinical response rate.

INTRODUCTION

Screening systems are naturally very important in the development of new anticancer agents, and the history of screening systems has been a story of trial and error in which attempts have been made to select better tumors for predicting clinical effectiveness in humans.

It is also well known that effective anticancer agents against animal tumors do not necessarily mean clinical effectiveness. Therefore, a screening system using human tumors has long been called for in the hope that it would

provide valuable new information on the discrepancies between clinical effectiveness and experimental data obtained from screening systems using animal tumors.

Therefore, since the successful xenotransplantation of human tumors into nude mice by Ryaard and Povlsen, the human tumor/nude mouse system has been expected to provide a valuable tool for the screning of anticancer agents.

In applying this system to anticancer agents, however, there are many problems to be solved. For example:

1. Are xenotransplants stable in growth rate, histology and function during successive transplantations in nude mice?

2. How can a typical human cancer line representative for a certain type of cancer (for example, stomach cancer) be selected? If this is impossible, how many lines are necessary to evaluate clinical effectiveness?

3. What is the most suitable dose of anticancer agents in nude mice to evaluate clinical effectiveness and what is the tissue distribution and toxicity of anticancer agents in nude mice?

Concerning the first problem, most human tumors have been reported to preserve their original nature, including histology, function and sensitivity to anticancer agents (Hata et al., 1982; Maruo et al., 1982). The growth rate has also been reported to be constant after a second serial xenotransplantation, although the initial xenotransplantation showed rather slow growth in nude mice (Povlsen et al., 1982). Based on these data, xenotransplanted human tumors appear to preserve their original characteristics although the possibility of selection at the initial xenotransplantation has not been completely ruled out because some tumors are liable to attack by high NK (natural killer) activity in nude mice (Habu et al., 1981) and other environmental effects due to species differences.

The second problem is very difficult to solve because it is well known that cancer in each patient shows different sensitivity to anticancer agents. To overcome this problem, we tested multiple human tumor lines for each type of cancer (for example, stomach cancer and lung cancer) and

calculated the response rates to compare them with the clinical response rates.

With respect to the third problem, we introduced the rational dose (RD) in addition to the maximum tolerated dose (MTD). RD is the dose which is necessary to maintain the mouse blood level the same as the human blood level in patients to obtain clinical effectiveness. Using this regimen, 11 lines of human stomach cancer, 13 lines of human lung cancer and six lines of human glioma are being treated with seven different anticancer agents.

This work is now in progress and interim data are given in this report.

MATERIALS AND METHODS

Animals

Six female BALB/cA-nu were used in each group. Nude mice were kept under specific pathogen free conditions throughout the experiment.

Human Cancer Xenografts

Profiles of human cancer xenografts are shown in Tables 1, 2 and 3.

Transplantation of Human Cancer

Approximately 8 mm^3 of fragments were asceptically xenotransplanted into 8-10 week-old female nude mice subcutaneously using a trocar.

Start of Treatment

When the cancer grew to 100-300 mm^3 in size, anticancer agents were administered.

Route of Administration

Intravenous administration was employed according to the route usually used for patients.

Table 1: Profiles of human gastric carcinoma xenografts

Tumor line	Histology	Prior therapy	Characteristics
SC-2-JCK	mucinous adenocarcinoma	-	c-myc ↑
SC-4-JCK	poorly-differentiated adenocarcinoma	+	
SC-6-JCK		-	SC
SC-7-JCK	moderately-differentiated adenocarcinoma	+	α_1AT
SC-9-JCK	well-differentiated adenocarcinoma	-	
St-4	poorly-differentiated adenocarcinoma	-	
ST-15	mucinous adenocarcinoma	-	AFP, CEA
ST-40	well-differentiated adenocarcinoma	-	
NS-3	poorly-differentiated adenocarcinoma	-	c-myc ↑, AFP, C_3, α_1AT
NS-8	moderately-differentiated adenocarcinoma	-	
4-1ST	poorly-differentiated adenocarcinoma	-	c-myc AFP, C_3

c-myc↑: amplification of c-myc (oncogene)
α_1AT: alpha-1-antitrypsin
CEA: carcinoembryonic antigen
SC: secretory component
AFP: alpha-fetoprotein
C_3: third component of complement

Table 2: Profiles of human lung carcinoma xenografts

Tumor line	Histology	Prior therapy	Characteristics
LX-1	small cell carcinoma	-	
Lu-24	small cell carcinoma	+	ACTH, ADH, MSH, CT
Lu-130	small cell carcinoma	+	ACTH, MSH
Lu-134	samll cell carcinoma	+	
Lu-116	large cell carcinoma	+	CSF
Lu-99	large cell carcinoma	-	CSF
Lu-65	large cell carcinoma	-	CSF
Lu-61	squamous cell carcinoma	-	CSF Ca↑
QG-56	squamous cell carcinoma	?	
LC-1-JCK(OTUK)	squamous cell carcinoma	-	CSF, Ca↑
LC-6-JCK	squamous cell carcinoma	-	CSF, Ca↑
L-27	adenocarcinoma	?	
LC-11-JCK	adenocarcinoma	-	

ACTH: adrenocorticotropic hormone
ADH: antidiuretic hormone
MSH: melanocyte stimulating hormone
CT: thyrocalcitonin
CSF: colony stimulating factor
Ca↑: hypercalcemia in the host nude mouse

Table 3: Profiles of human glioma xenografts

Tumor line	Histology	Prior therapy	Characteristics
Epe-1-JCK	ependymoma	-	
GL-1-JCK	ependymoma	radiation	
GL-2-JCK	glioblastoma multiforme	radiation	
GL-3-JCK	glioblastoma multiforme	-	
GL-5-JCK	glioblastoma multiforme	-	hypervolemia in host nude mouse
GL-8-JCK	glioblastoma multiforme	?	

Dose

MTD and RD (RD is explained below).

Schedule of Administration

As a rule, single injection administration was used. Antimetabolities were administered once a day five times. ACNU was administered following the method shown below.

Formula to Calculate Tumor Volume

$1/2 \times \text{length} \times \text{width}^2$

Time of Evaluation

2 weeks after starting treatment.

Evaluation

1. Mann-Whitney test, U-test ($P<0.01$)

2. Tumor reduction rate [T/C(Tested tumor weight/ Control tumor weight) < 50%].

Anticancer agents

Mitomycin C (MMC)	Cyclophosphamide (CPM)
ACNU	Adriamycin (ADM)
Vincristine (VCR)	5-fluorouracil (5-FU)
Methotrexate (MTX)	

Determination of MTD

Doses of anticancer agents were increased stepwise at a ratio of 1.2 starting from an arbitrary amount in six nude mice in each group. The MDT was determined as the highest dose at which no deaths were observed within one month after treatment.

Determination of RD

Various doses of anticancer agent (for example, MTD, 1/2 MTD, 1/4 MTD and 1/8 MTD) were administered to six nude mice in each group. Blood was sampled, heparinized, centrifuged and preserved at -20°C. Blood levels of each anticancer agent were assayed and compared to the clinical data already reported in the literature, and the RD was determined.

Assay Method for Each Anticancer Agent

MMC. Bioassay using the thin layer cup method (Miyamura et al., 1950).

CPM. 4-OH CPM assayed according to Hohorst (Hohorst et al., 1976; Wagner et al., 1981).

ACNU. High performance liquid chromatography (HPLC) assay after extraction by 1.2-dichroethanol (Nakamura et al., 1977).

ADR. RD determined from data already reported (Johansen, 1981).

VCR. Method using ^{3}H-labeled Vincristine (Jackson, et al., 1978).

5-FU. Bioassay using Staphylococcus aureus (Anada et al., 1974).

MTX. Method of Bertino and Fisher (Bertino et al., 1964).

RESULTS

Determination of MTD

Results of experiments to determine the MTD are shown in Table 4.

Table 4

Maximum tolerated dose, rational dose and clinical dose

Drugs	Nude mice					Clinical Dose	
	MTD		RD				
	mg/kg	mg/m^2	mg/kg	mg/m^2		mg/kg	mg/m^2
Mitomycin C	6.7	(20)	1.7	(5.1)	=1/4MTD	0.17	(6.2)
Vincristine	1.6	(4.8)	0.4	(1.2)	=1/4MTD	0.03	(1.2)
Cyclophosphamide	260	(780)	33-16	(99-48)	=1/8MTD	20	(750)
ACNU	48	(144)	10	(30)	=1/5MTD	2.5	(94)
			0 min 8 25 min 2 70 min 0.8				
5-FU	19 x 5	(57)	RD>MTD			10	(375)
Methotrexate	15 x 5	(45)	RD>MTD			0.5	(17)
Adriamycin	12	(36)	12	(36)	=MTD	1.6	(60)

Determination of RD

The time course of blood VCR level after injection is shown in Figure 1. Blood levels after a single administration of 0.4 mg/kg VCR to nude mice simulated closely those in the blood of humans administered 0.3 mg/kg of VCR. Therefore, the RD of VCR was estimated as 0.4 mg/kg (1/4 MTD).

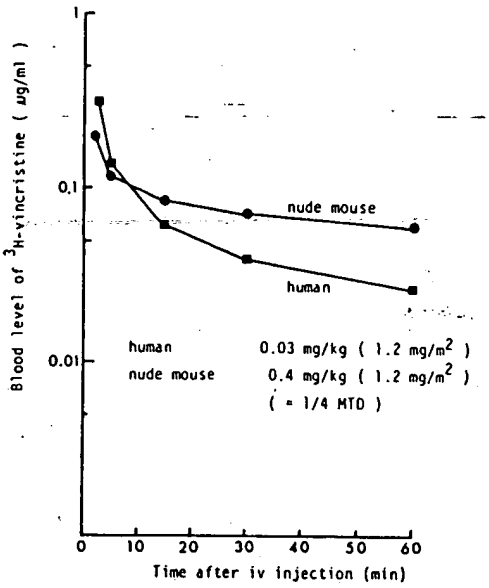

Figure 1. Time course of ^{3}H-vincristine blood levels in humans and nude mice.

In case of ACNU, since the blood level showed a rapid decrease in nude mice after a single injection as shown in Figure 2, repeated injections were necessary to simulate the human blood level as shown in Figure 3. Therefore, the protocol of administration for treatment with the RD of ACNU was determined to give 8 mg/kg at 0 minute, 2 mg/kg at 25 minutes and 0.8 mg/kg at 70 minutes. The RD values for each anticancer agent thus determined are shown in Table 4. The ratio of RD to MTD varied for each drug as follows: The RD of MMC and VCR was about 1/4 MTD. The RD

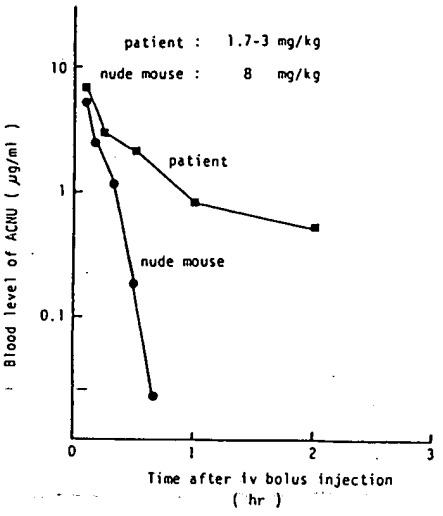

Figure 2. Time course of ACNU blood levels in human patients and nude mice.

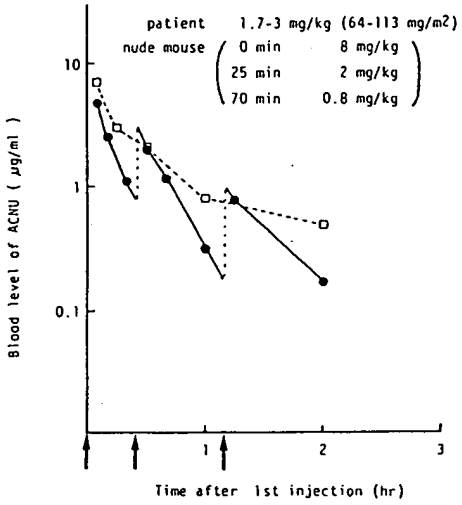

Figure 3. Time course of ACNU blood levels in humans and nude mice when injected repeatedly.
□ ---□ : human patients ●——● : nude mice
↑ : injection

of CPM was about 1/8 MTD and that of ACNU about 1/5 MTD. The RD of ADM was almost the same as the MTD. On the other hand, blood levels of 5-FU MTX did not reach the MTD of the patient as shown by RD>MTD although the difference was not very great.

Results of Treatment of Stomach Cancer

As summarized in Table 5, the response rate was much higher than the clinical response rate when tumors were treated by the MTD of various anticancer agents. It is noteworthy that VCR was ineffective against all stomach cancers even treated with the MTD.

As shown in Table 6, the response rate for the RD was generally lower than that of the MTD treated group.

A comparison of response rates among groups treated with MTD and RD, and the clinical response rate is shown in Table 7. The response rate of the group administered the RD was almost comparable to the clinical response rate although the experiments have not been completed.

Tumor phenotypes such as the histologic type, production of human serum proteins and c-myc oncogene amplification seemed to have no apparent relation to the responsiveness of stomach cancers to anticancer agents.

Generally speaking, although the response rates at the RD were much less than those at the MTD, T/C values were rather similar in the two groups.

Results of Treatment of Lung Cancers

A summary of treatment of non-small-cell lung carcinoma by the MTD is shown in Table 8. Most lung cancers except for adenocarcinoma show good response to many types of anticancer agents.

When non-small-cell lung carcinomas were treated with the RD, many anticancer agents became ineffective (Table 9). A comparison of responsiveness is shown in Table 10. The response rates to the RD of ACNU, 5-FU and MTX were almost the same as the clinical response rates. Responses

Table 5

Treatment of human gastric carcinoma xenografts by MTD of anticancer agents

Drug	Relative tumor growth (T/C %)											Response rate (%)
	SC-2	SC-4	SC-6	St-4	NS-3	NS-8	4-lst	SC-7	SC-9	St-40	St-15	
MMC	(21)	(38)	(5)	62	(27)	83	(17)	(39)	(12)	(19)	(23)	82 (9/11)
CPM	88	86	81	107	69	71	(48)	92	96	59	(46)	18 (2/11)
ACNU	(40)	77	(3)	(42)		88	(2)	81	(35)		(32)	67 (6/ 9)
ADR	76	51	87	72	(27)	91	51	58	(37)	63	88	18 (2/11)
VCR	70	70	92	72	92	90	78	92	46	64	88	0 (0/11)
5-FU	(40)	83	58	(49)	63	66	70	74	61	(42)	72	27 (3/11)
MTX	67	101	78					96		68	109	0 (0/ 6)

( ) : Effective Blank columns: not tested

Table 6

Treatment of human gastric carcinoma xenografts by RD of anticancer agents

Drug	Relative tumor growth (T/C %)											Response rate (%)
	SC-2	SC-4	SC-6	St-4	NS-3	NS-8	4-1st	SC-7	SC-9	St-40	St-15	
MMC	84	66	(30)	78	110	92	57	81	97	52	(32)	18 (2/11)
CPM	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)		0 (0/ 9)
ACNU	65	81				(-)		(98)			83	0 (0/ 5)
ADR	76	51	87	72	(27)	91	51	58	(37)	63	88	18 (2/11)
VCR	79	72	66	66		102	87	96	87	74	102	0 (0/10)
5-FU	(40)	83	58	(49)	63	66	70	74	61	(42)	72	27 (3/11)
MTX	67	101	78					96		68	109	0 (0/ 6)

( ) : Effective (-) : Ineffective from MTD data

blank columns : not tested

Table 7

Response rate of human gastric carcinoma xenografts to anticancer agents

Anticancer agent	Response rate (%)		Clinical response rate (%)
	MTD	RD	
MMC	82 (9/11)	18 (2/11)	30
CPM	18 (2/11)	0 (0/ 9)	6
ACNU	67 (6/ 9)	0 (0/ 5)	11
ADR	18 (2/11)	18 (2/11)	18
VCR	0 (0/11)	0 (0/10)	0
5-FU	27 (3/11)	27 (3/11)	23
MTX	0 (0/ 6)	0 (0/ 6)	?

Table 8

Treatment of human non-small-cell lung carcinoma xenografts by MTD of anticancer agents

Drug	Relative tumor growth (T/C %)									Response rate (%)
	Lu-116	Lu-99	Lu-65	Lu-61	QG-56	LC-1	LC-6	L-27	LC-11	
MMC	(5)	(12)	(5)	(14)	(39)	(21)	(1[3]*)	52	(43)	89 (8/9)
CPM	(8)	(10)	(4)	61	64	(31)	(21)	57	71	56 (5/9)
ACNU	(0[6])	(25)	(2)	(13)	58		(0[6])	57	76	63 (5/8)
ADR	(35)	72	(47)	78	55	(50)	(31)	59	63	44 (4/9)
VCR	(31)	(18)	(27)	86	99	58	(40)	72	85	44 (4/9)
5-FU		(46)	70	105	72	66	100	91	79	13 (1/8)
MTX			83	100		97	(38)	105	92	17 (1/6)

( ) : Effective * : Number of cured mice

Table 9

Treatment of human non-small-cell lung carcinoma xenografts by RD of anticancer agents

Drug	Relative tumor growth (T/C %)									Response rate (%)
	Lu-116	Lu-99	Lu-65	Lu-61	QG-56	LC-1	LC-6	L-27	LC-11	
MMC	109	64	65	61	72	70	87	(-)	92	0 (0/8)
CPM	114	66	80	(-)	76		102	(-)	(-)	0 (0/8)
ACNU	(9)				97			(-)	(-)	25 (1/4)
ADR	(35)	72	(47)	78	55	(50)	(31)	59	63	44 (4/9)
VCR	72	58	76	(-)	(-)	86	79	(-)	(-)	0 (0/9)
5-FU		(46)	70	105	72	66	100	91	79	13 (1/8)
MTX			83	100		97	(38)	105	92	17 (1/6)

(○) : Effective

(-): Ineffective from MTD data

Table 10.

Response rate to human non-small-cell lung carcinoma xenografts to anticancer agents

Anticancer agent	Response rate (%)		Clinical response rate (%)
	MTD	RD	
MMC	90 (9/10)	0 (0/ 8)	13 - 26
CPM	56 (5/ 9)	0 (0/ 8)	8 - 23
ACNU	63 (5/ 8)	25 (1/ 4)	13
ADR	44 (4/ 9)	44 (4/ 9)	13 - 14
VCR	40 (4/10)	0 (0/10)	10 - 19
5-FU	13 (1/ 8)	13 (1/ 8	9 - 13
MTX	17 (1/ 6)	17 (1/ 6)	10 - 21

to MCC, CPM and VCR at the RD were lower than clinical response rates. Conversely, the response rate to ADR was slightly higher than the clinical response rate.

Comparing Tables 8 and 9, the difference in T/C between the MTD and RD treated groups was much higher than that for stomach cancers.

Response rates of small cell carcinoma are tentatively shown in Table 11, although it seems too early to draw any conclusions on the response rates because only a few small-cell carcinomas have been tested.

Results of Glioma Treatment

A summary of the treatment of gliomas is shown in Table 12. The repsonse rate was not compared to the clinical response rate because there were only a few tumor lines tested, and the blood brain barrier must be taken into account in the clinical treatment of gliomas. It was noteworthy that the patterns of effectiveness to anticancer agents were different from those of stomach cancer, i.e., VCR was effective against glioma xenografts but was totally ineffective against stomach cancer. On the other hand, MMC was not effective against gliomas but effective against most stomach cancers.

DISCUSSION

Although studies are now in progress and the data are incomplete, the above results may provide some valuable information.

Prediction of Clinical Response Rate

As shown in Table 7, response rates of stomach cancer xenografts treated with the RD were almost the same as the clinical response rate. Therefore, this system is thought to be a useful tool in predicting the clinical response rate of new anticancer agents.

Some discrepancies noticed between experimental and clinical response rates of lung cancers to anticancer

Table 11

Response rate of human small cell lung carcinoma xenografts to anticancer agents

Anticancer agent	Response rate (%)		Clinical response rate (%)
	MTD	RD	
MMC	100 (4/4)	75 (3/4)	37
CPM	25 (1/4)	0 (0/3)	28 - 51
ACNU	100 (4/4)	75 (3/4)	47
ADR	75 (3/4)	75 (3/4)	25 - 31
VCR	75 (3/4)	0 (0/4)	33 - 56
5-FU	0 (0/3)	0 (0/3)	12
MTX	0 (0/1)	0 (0/1)	30 - 39

Table 12

Response rate of human glioma xenografts to anticancer agents

Anticancer agent	Response rate (%)	
	MTD	RD
MMC	50 (3/6)	0 (0/6)
CPM	100 (5/5)	0 (0/5)
ACNU	100 (5/5)	(0/3)
ADR	80 (4/5)	80 (4/5)
VCR	100 (6/6)	50 (3/6)
5-FU	0 (0/6)	0 (0/6)
MTX	17 (1/6)	17 (1/6)

agents (Table 10) may be partly due to inadequate numbers and types of cancers tested. In particular, the difference in the spectrum of histologic types from clinical cases might be important in lung cancer since histologic types are highly variable. Therefore, the response rate must be evaluated for each histologic type of cancer if possible. The poor responsiveness of adenocarcinoma shown in Table 9 suggests the possibility of the above assumption. We are now planning to add more human lung cancer lines and replace unsuitable lines.

Although more detailed studies must be performed, this human tumor/nude mouse system is thought to be useful for the stage following Phase I studies in developing new anticancer agents where blood levels of the new agents have already been determined. The data obtained from this system at that stage will provide useful information for progress to Phase II studies. For example, it may be possible to administer the new drug to patients with special types of cancers which are anticipated to be sensitive to the agent according to the data obtained from this system.

Different Patterns of Effectiveness among Types of Cancers

In a comparison of the effectiveness of anticancer agents against stomach cancers and gliomas, different patterns became apparent, i.e., VCR was totally ineffective against stomach cancers but effective against gliomas. On the other hand, MMC and 5-FU were ineffective against gliomas but effective against stomach cancers although the RD of 5-FU was higher than the MTD. These data strongly suggest that human cancer has different sensitivity to certain anticancer agents usually depending on origin and histologic type, although individual cancers show some minor differences in responsiveness. This information may be useful in estimating the spectrum of tumors sensitive to a new anticancer agent.

MTD and RD

The difference in responsiveness between groups administered the MTD and RD was higher in lung cancers than that in stomach cancers as shown in Tables 5, 6, 8 and 9. These data suggest that most lung cancers, except for adenocarci-

noma, show sensitivity to many anticancer agents if the dose is adequate. In contrast to lung cancer, most stomach cancers showed no marked improvement if a higher dose was given. These data suggest that high dose chemotherapy may be effective against lung cancers but not so effecitve against stomach cancers.

Finally, it may also be important to select nude mouse strains suitable for experimental chemotherapy. One of desirable trains is a higher tolerance to 5-FU and MTX, because the RD values of these drugs are higher than the MTD and the effects of these drugs in nude mice may be underestimated. Another anticipated character of this tumor host is more rapid and constant human tumor growth. Studies are now in progress at the Central Institute for Experimental Animals to improve nude mice.

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EMERGING MODELS IN THE U.S.A.: SWINE, WOODCHUCKS, AND THE HAIRLESS GUINEA PIG

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ABSTRACT

Swine have been used in biomedical research for many years, but have generally been limited to those locations with personnel familiar with this species and with specially designed facilities and equipment. There is currently a growing trend in the United States for more swine, both miniature and domestic to be used as research models. Commercial availability, education through workshops and symposia, and specific research applicability in the areas such as: organ transplantation, cardiovascular surgery, mutrition, diabetes, dermatology, and renal physiology have all contributed to the increased usage of swine. Additionally, increasing costs and public concern about the use of random source dogs and cats have also resulted in a refocus on swine as a laboratory animal model.

The woodchuck (Marmota monax) has recently gained a role as a laboratory animal model when it was discovered that woodchuck hepatitis virus (WHV) is closely related to hepatitis B virus in humans (HBV). Chronic infections in woodchucks with WHV have shown protein particles in their blood which are similar to the Australian antigen found on the surface of HBV. There is also immunologic response similarities by the respective host to these viruses. These findings have resulted in a number of laboratories using the woodchuck in infectious disease comparative research studies.

A euthymic hairless guinea pig has been described in